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Attenuation of morphine withdrawal symptoms by subtype-selective metabotropic glutamate receptor antagonists

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1 We have previously shown that chronic antagonism of group I metabotropic glutamate receptors (mGluRs), in the brain, attenuates the precipitated morphine withdrawal syndrome in rats. In the present investigation we assessed the effects of chronic antagonism of group II and III mGluRs on the severity of withdrawal symptoms in rats treated chronically with subcutaneous (s.c.) morphine.

2 Concurrently with s.c. morphine we infused intracerebroventricularly (i.c.v.) one of a series of phenylglycine derivatives selective for specific mGluR subtypes. Group II mGluRs ($mGluR_{2,3}$), which are negatively coupled to adenosine 3′: 5′-cyclic monophosphate (cyclic AMP) production, were selectively antagonized with 2s, 1′s, 2′s-2-methyl-2-(2′-carboxycyclopropyl) glycine (MCCG). Group III mGluRs ($mGluR_{4,6,7\ and\ 8}$), which are also negatively linked to cyclic AMP production, were selectively antagonized with α-methyl-L-amino-4-phosphonobutanoate (MAP4). The effects of MCCG and MAP4 were compared with α-methyl-4-carboxyphenylglycine (MCPG), which non-selectively antagonizes group II mGluRs, as well as group I mGluRs ($mGluR_{1,5}$) which are positively coupled to phosphatidylinositol (PI) hydrolysis.

3 Chronic i.c.v. administration of both MCCG and MAP4 significantly decreased the time spent in withdrawal, MCPG and MCCG reduced the frequency of jumps and wet dog shakes and attenuated the severity of agitation.

4 Acute i.c.v. injection of mGluR antagonists just before the precipitation of withdrawal failed to decrease the severity of abstinence symptoms. Rather, acute i.c.v. injection of MCCG significantly increased the time spent in withdrawal.

5 Our results suggest that the development of opioid dependence is affected by mGluR-mediated PI hydrolysis and mGluR-regulated cyclic AMP production.

Keywords: Opioid; morphine; metabotropic glutamate receptor; dependence; α-methyl-4-carboxyphenylglycine (MCPG); 2s, 1′s, 2′s-2-methyl-2-(2′-carboxycyclopropyl)-glycine (MCCG); α-methyl-L-amino-4-phosphonobutanoate (MAP4)

Introduction

Although opioid analgesics are widely used in the management of pain, repeated use may lead to the development of tolerance and dependence. Tolerance is indicated by a decreased efficacy of the drug after chronic use, thereby leading to the requirement for a higher dose to achieve the desired analgesic effect. Dependence is a continued need for the drug to maintain a state of physiological equilibrium, following repeated administration, and is evidenced by withdrawal symptoms when drug administration is terminated. Recent evidence supports the involvement of excitatory amino acid (EAA), or glutamate, receptors in the development of tolerance and dependence (Marek *et al*., 1991a,b; Trujillo & Akil, 1991; Fundytus & Coderre, 1994). Specifically, we have previously shown that chronic i.c.v. administration of antagonists selective for metabotropic glutamate receptors (mGluRs) significantly attenuates the development of dependence to systemically administered morphine (Fundytus & Coderre, 1994).

Metabotropic glutamate receptors are directly linked to intracellular second messenger systems via guanine nucleotide regulatory proteins (G proteins; Sladeczek *et al*., 1985; Sugiyama *et al*., 1987). Several subtypes of mGluRs, from $mGluR_1$ to $mGluR_8$, have been cloned (Nakanishi, 1992; Schoepp & Conn, 1993; Okamoto *et al*., 1994; Saugstad *et al*., 1994; Duvoisin *et al*., 1995). The subtypes of mGluRs can be divided into groups based on receptor pharmacology, signalling cascades and sequence similarities (Hayashi *et al*., 1994). The first group of mGluRs consists of $mGluR_1$ and $mGluR_5$, which are positively linked to the phosphatidylinositol second messenger system (Schoepp & Conn, 1993; Hayashi *et al*., 1994). Activation of these receptors leads to phospholipase C (PLC)-mediated phosphatidylinositol (PI) hydrolysis. The second group of mGluRs consists of $mGluR_2$ and $mGluR_3$, which are negatively coupled, via adenylate cyclase, to the production of adenosine-3′:5′-cyclic monophosphate (cyclic AMP) (Hayashi *et al*., 1994). The third group of mGluRs consist of $mGluR_{4,6,7\ and\ 8}$, which are also negatively coupled to cyclic AMP production, but which show a different receptor pharmacology than $mGluR_{2,3}$ in that they are selectively activated by L-amino-4-phosphonobutanoate (L-AP4) (Hayashi *et al*., 1994; Saugstad *et al*., 1994).

In a previous study (Fundytus & Coderre, 1994), we showed that chronic intracerebroventricular (i.c.v.) infusion of the mGluR antagonist (*S*)-4-carboxyphenylglycine ((*S*)-4C-PG) concurrently with systemic morphine attenuated the development of morphine dependence. Although (*S*)-4C-PG selectively antagonizes group I mGluRs ($mGluR_1$ and $mGluR_5$), it has a secondary effect whereby it activates group II mGluRs ($mGluR_2$, $mGluR_3$) (Eaton *et al*., 1993; Hayashi *et al*., 1994; Watkins & Collingridge, 1994). It is therefore not entirely clear whether antagonism of $mGluR_{1,5}$ or activation of $mGluR_{2,3}$ is primarily responsible for the ability of (*S*)-4C-PG to attenuate the development of morphine dependence. To examine the relative contribution of each of the mGluR subtypes, we chose a range of phenylglycine antagonists selective to specific mGluRs. We examined how non-selective antagonism of group I ($mGluR_1$ and $mGluR_5$) and group II ($mGluR_2$ and $mGluR_3$) subtypes would affect the development of morphine dependence by administering α-methyl-4-carboxyphenylglycine (MCPG) (Eaton *et al*., 1993; Hayashi *et al*.,

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1994; Thomsen *et al*., 1994) i.c.v. concurrently with systemic morphine. We also selectively antagonized $mGluR_2$ and $mGluR_3$ with 2s,1′s,2′S-2-methyl-2-(2′carboxycyclopropyl)-glycine(MCCG) (Jane *et al*., 1994) and $mGluR_{4,\ 6-8}$ receptors with α-methyl-L-amino-4-phosphonobutanoate (MAP4) (Jane *et al*., 1994), respectively. Furthermore, the effects of chronic treatment with these mGluR antagonists on morphine dependence were compared with the effects of acute treatment with the same agents given 10 min before naloxone. In the present study, we showed that chronic non-selective antagonism of mGluRs with MCPG, and chronic selective antagonism of either group II or III mGluRs significantly attenuates the development of morphine dependence.

Methods

Subjects and surgery

Subjects were male Long Evans hooded rats (Charles River, Quebec) weighing 280–350 g at the time of surgery. Rats were housed in groups of 2 to 4, maintained on a 12:12 h light:dark cycle (lights on at 06 h 00 min), and given food and water *ad libitum*.

On Day 0 rats were anaethestized with sodium pentobarbitone (Somnotol, MTC Pharmaceuticals, 60 mg kg^{-1}), and a 23 gauge stainless steel cannula was implanted stereotaxically in the lateral vertical of each rat (AP = −1.3 mm and L = −1.8 mm from bregma, and V = −3.8 mm (for chronic infusion) or V = −3.0 mm (for acute i.c.v. injections) from the top of the skull; Paxinos & Watson, 1986). For rats given chronic i.c.v. treatment, the cannula was attached to a Model 2001 Alzet osmotic pump filled with one of the antagonists or vehicle (dilute NaOH/saline). While the rats were still under pentobarbitone anaesthesia, one unprimed (not yet pumping) Model 2ML1 Alzet osmotic pump containing 50 mg ml^{-1} morphine sulphate (gift from Sabex, Quebec) solution was implanted subcutaneously (s.c.) on the back of each rat. Infusion of morphine began approximately 2 to 4 h following pump implantation. On the following day, Day 1, rats were briefly anaesthetized with halothane and a second unprimed Model 2ML1 Alzet pump containing 70 mg ml^{-1} morphine sulphate solution was implanted s.c. on the back of each rat. Once both pumps were in place, morphine sulphate was continuously infused s.c. at a rate of 10 μl h^{-1} from each pump, for a total dose of 36.65 μmol day^{-1} (28.8 mg day^{-1}). This two day pump implantation procedure was used to reduce the risk of mortality due to the accumulation of lethal systemic morphine concentrations before any tolerance development. Concurrently with morphine, the mGluR antagonists, MCPG ($n = 20$), MCCG ($n = 18$), MAP4 ($n = 17$) (Tocris Cookson, Bristol, U.K.) or vehicle ($n = 18$) were continuously infused at a rate of 1 μl h^{-1} in a dose of either 1.6, 8 or 40 nmol day^{-1} intracerebroventricularly (i.c.v.) in rats treated chronically with mGluR antagonists. To assess the effects of chronic administration of the selective mGluR antagonists on general (non-withdrawal) behaviour in rats not dependent on morphine, some rats were given 7 days of i.c.v. vehicle or 40 nmol day^{-1} of either MCPG ($n = 3$), MCCG ($n = 4$) or MAP4 ($n = 4$) without concurrent morphine treatment. The effects of chronic administration of selective mGluR antagonists were also compared with effects of acute administration of the antagonists given as a single i.c.v. injection 10 min before the induction of withdrawal. Acute i.c.v. injections of either MCPG ($n = 5$), MCCG ($n = 6$) or MAP4 ($n = 6$) were given in a dose of 2 nmol 4 μl^{-1}, or 4 μl vehicle ($n = 15$), to rats that received chronic systemic morphine treatment as described above. A dose of 2 nmol was chosen to approximate the level received over a 1 to 2 h period in rats treated chronically with 40 nmol day^{-1}. Doses as high as 99 nmol i.c.v. have been used in learning experiments with no obvious side effects (Riedel & Reymann, 1993). The effects of acute administration of mGluR antagonists on general (non-withdrawal) behaviour were assessed by observing the behaviour of non-dependent rats after a single i.c.v. injection of either MCPG ($n = 4$), MCCG ($n = 4$) or MAP4 ($n = 4$).

Measurement of withdrawal and non-withdrawal behaviours

Precipitated abstinence symptoms were assessed on the seventh day of treatment after injection of the opioid antagonist naloxone. Naloxone hydrochloride (Research Biochemicals Inc., Natick, MA) was injected s.c. in a volume of 1 ml kg^{-1} for a dose of 1 mg kg^{-1}. In chronically treated rats, behaviour was observed for 10 min before and 40 min after naloxone injection, during which time withdrawal symptoms were assessed by measuring the amount of time spent teeth chattering and writhing, as well as by counting jumps and wet dog shakes. In rats given an acute i.c.v. injection of mGluR antagonists, withdrawal behaviours were assessed for 10 min before the i.c.v. injection, 10 min after i.c.v. injection but before naloxone, and for 40 min after the injection of naloxone. For both chronic and acute conditions, agitation was assessed by rating the severity of vocalization upon light brushing of the back of the neck at both 20 and 40 min after the injection of naloxone. Severity of vocalization was rated on a scale of 0 to 3 where 0 = absent and 3 = severe. Also, for both chronic and acute i.c.v. treatment conditions, the time spent in withdrawal and non-withdrawal behaviours (ambulating, rearing, grooming and resting) was also measured for comparison in non-dependent rats (not given morphine) and morphine-dependent rats (given chronic s.c. morphine).

Statistical analysis

Timed withdrawal behaviours (teeth chattering, writhing) were analysed by 1 way ANOVA comparing the effects of various doses of each drug with the vehicle control group. Since all drugs were dissolved in the same vehicle, a single control group was used for each experiment to minimize the number of animals exposed to the full-fledged opioid withdrawal syndrome. Testing of the vehicle-treated rats was spread across the testing days for the experimental animals. Significant effects were further analysed by use of *post-hoc* LSD *t* tests. Counted withdrawal behaviours (number of jumps and wet dog shakes) and severity of agitation were analysed by a Kruskal-Wallis ANOVA for non-parametric data, followed by Mann-Whitney U-tests on significant main effects.

The effect of chronic antagonism of subtypes of mGluRs on non-withdrawal behaviours (ambulating, rearing, grooming and resting) was assessed by comparing the first two time blocks (i.e. 10 min before naloxone injection and 10 min after naloxone injection) for rats in each treatment group. The effects of acute i.c.v. injection of mGluR antagonists was assessed by comparing non-withdrawal and withdrawal behaviours for the first three times blocks (i.e. 10 min before i.c.v. injection, 10 min after i.c.v. injection but before naloxone injection, and 10 min after naloxone injection) in non-dependent and morphine-dependent rats. In both cases, a 3-way mixed ANOVA with i.c.v. treatment and morphine treatment as independent variables and time block as a repeated measure was performed on the % of time spent in each behaviour. Significant effects were further analysed with *post-hoc* LSD *t* tests.

Results

Figure 1 illustrates the severity of abstinence symptoms during the 40 min withdrawal period in rats chronically infused with s.c. morphine and either vehicle, MCPG, MCCG or MAP4 i.c.v. This experiment was performed to determine if chronic blockade of mGluRs would attenuate the development of morphine dependence. Chronic s.c. administration of 36.65 μmol day^{-1} morphine sulphate resulted in an intense

and reliable withdrawal syndrome, evidenced by the occurrence of teeth chattering, writhing, jumping, wet dog shaking and vocalization on touch (agitation) in vehicle-treated rats.

Figure 1a shows the amount of time spent in withdrawal (teeth chattering and writhing combined) during the 40 min withdrawal period for morphine-dependent rats treated concurrently with either i.c.v. vehicle, or 1.6, 8 or 40 nmol day^{-1} of either MCPG, MCCG or MAP4. ANOVA indicated a significant effect of MCCG ($F_{(3,32)}=4.14$, $P<0.05$) and MAP4 ($F_{3,31)}=4.62$, $P<0.05$), but not MCPG ($F_{3,34)}=1.90$, $P>0.05$). Both MCCG and MAP4 significantly decreased the time spent in withdrawal, as compared to the vehicle control group, at a dose of 1.6 nmol day^{-1}. Only MCCG was effective at 8 nmol day^{-1}, and only MAP4 was effective at 40 nmol day^{-1}. Although there was a trend for the non-selective mGluR antagonist MCPG to reduce the time spent in withdrawal, this effect failed to reach statistical significance.

Figure 1b illustrates the average frequency of counted symptoms (jumps and wet dog shakes combined) during the 40 min withdrawal period for morphine-dependent rats. Kruskal-Wallis ANOVA for non-parametric data indicated a significant effect of MCPG ($H_{(3,38)}=11.74$, $P<0.05$) and MCCG ($H_{(3,36)}14.14$, $P<0.01$), but not MAP4 ($H_{(3,35)}=6.08$, $P>0.05$). As indicated, MCPG significantly decreased the frequency of counted symptoms compared to the vehicle-treated control group at all doses used. MCCG significantly decreased the occurrence of counted symptoms at the highest dose, 40 nmol day^{-1}. Although there was a trend for MAP4 to decrease the frequency of control symptoms as dose was increased, this effect failed to reach statistical significance.

Figure 1c shows the average severity of agitation, as indicated by vocalization upon being lightly touched on the back of the neck, for morphine-dependent rats tested at 20 and 40 min post-naloxone. Kruskal-Wallis ANOVA for non-parametric data indicated a significant effect of MCPG ($H_{(3,38)}=11.65$, $P<0.05$), but not MCCG ($H_{(3,36)}=7.64$, $P>0.05$) nor MAP4 ($H_{(3,35)}=3.36$, $P>0.05$). The non-selective antagonist MCPG significantly decreased the severity of agitation at all doses used. Although MCCG appeared to attenuate the severity of agitation at 40 nmol day^{-1}, Kruskal-Wallis ANOVA failed to reach statistical significance. MAP4 did not significantly affect agitation at any of the doses used.

To verify that chronic infusion of mGluR antagonists had limited effects on general behaviour, non-withdrawal and withdrawal behaviours were compared during the 10 min before naloxone injection and the 10 min after naloxone injection for non-dependent and morphine-dependent rats chronically infused i.c.v. with either vehicle or 40 nmol day^{-1} of MCPG, MCCG or MAP4. Statistics confirmed that rats were more active earlier in the test session, with more time spent ambulating, rearing and grooming and less time spent resting, regardless of i.c.v. treatment (LSD t test, $P<0.05$). Before the injection of naloxone non-dependent and morphine-dependent rats behaved very similarly. After the injection of naloxone, non-dependent rats spent more time in non-withdrawal, and less time in withdrawal, behaviours than morphine-dependent rats (LSD t test, $P<0.05$). There were only a few effects of i.c.v. treatment on general activity level. Regardless of morphine treatment, i.c.v. MCPG- and MAP4-treated rats ambulated more than i.c.v. vehicle-treated rats ($P<0.05$, LSD t test). Also, non-dependent i.c.v. MCCG- and MAP4-treated rats reared more than non-dependent i.c.v. vehicle-treated rats ($P<0.05$, LSD t test) (data not shown).

Figure 2 depicts withdrawal symptoms during the 40 min withdrawal period for morphine-dependent rats given an acute i.c.v. injection of either vehicle or 2 nmol of either MCPG, MCCG or MAP4 10 min before naloxone injection. This experiment was performed to determine if acute blockade of mGluRs would decrease the expression of abstinence symptoms once dependence had developed.

Figure 2a illustrates the time spent in withdrawal (teeth chattering and writhing combined) during the 40 min withdrawal period for dependent rats given an acute i.c.v. injection

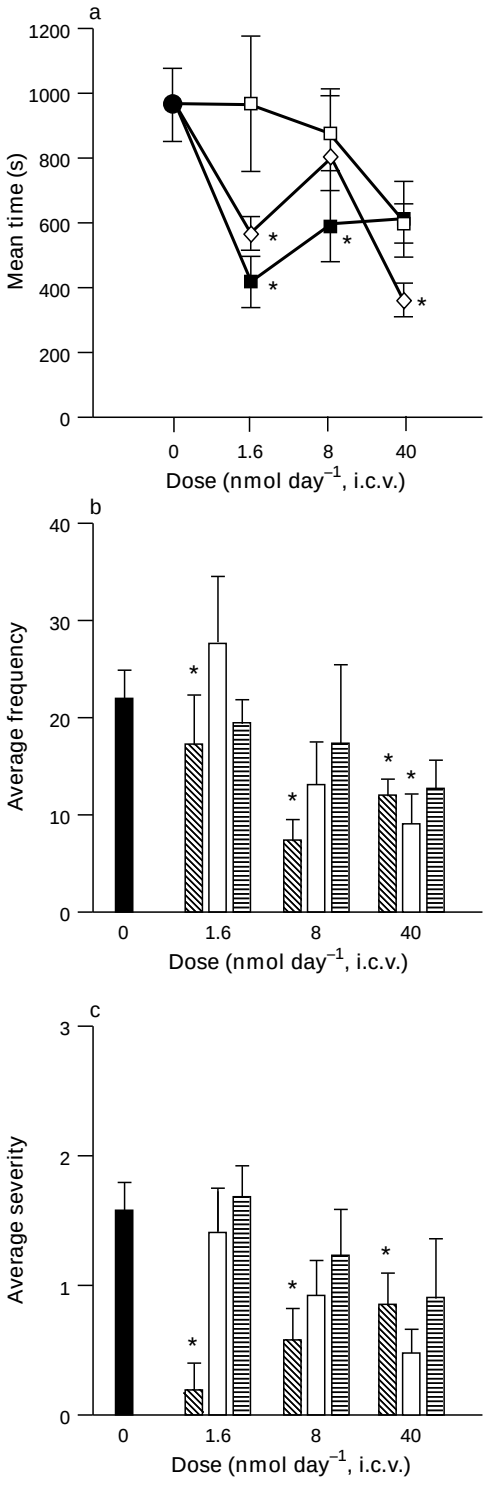

Figure 1 (a) Mean time spent in withdrawal (teeth chattering and writhing combined) during the 40 min withdrawal period for morphine-dependent rats treated chronically with either vehicle (●), MCPG (□), MCCG (■) or MAP4 (◇) i.c.v. Vertical lines show s.e.mean. *Significantly less than control ($P<0.05$, LSD t test). (b) Average frequency of counted symptoms (jumps and wet dog shakes combined) during the 40 min withdrawal period for morphine-dependent rats given either vehicle solid columns, MCPG diagonally-hatched columns, MCCG open columns or MAP4 horizontally-hatched columns. *Significantly less than control ($P<0.05$, Mann-Whitney U test). (c) Average severity of agitation during withdrawal for morphine-dependent rats given either vehicle solid columns, MCPG diagonally-hatched columns, MCCG open columns or MAP4 horizontally-hatched columns. *Significantly less than control ($P<0.05$, Mann-Whitney U test).

of either vehicle or 2 nmol of either MCPG, MCCG or MAP4. ANOVA indicated a significant effect of i.c.v. treatment ($F_{(3,28)}=4.20$, $P<0.01$). Acute injection of 2 nmol of MCCG 10 min before the precipitation of withdrawal significantly increased the time spent teeth chattering and writhing.

Figure 2b shows the average frequency of counted symptoms (jumps and wet dog shakes combined) during the 40 min withdrawal period for dependent rats given an acute i.c.v. injection of either vehicle, MCPG, MCCG or MAP4. Krusal-Wallis ANOVA for non-parametric data indicated that there were no differences between vehicle-treated rats and mGluR antagonist-treated rats ($H_{(3,32)}=0.40$ $P>0.05$).

Figure 2c shows the severity of agitation for dependent rats given an acute i.c.v. injection. Again, Krusal-Wallis ANOVA for non-parametric data indicated that there were no differences between vehicle-treated and mGluR antagonist-treated rats ($H_{(3,32)}=1.84$, $P>0.05$).

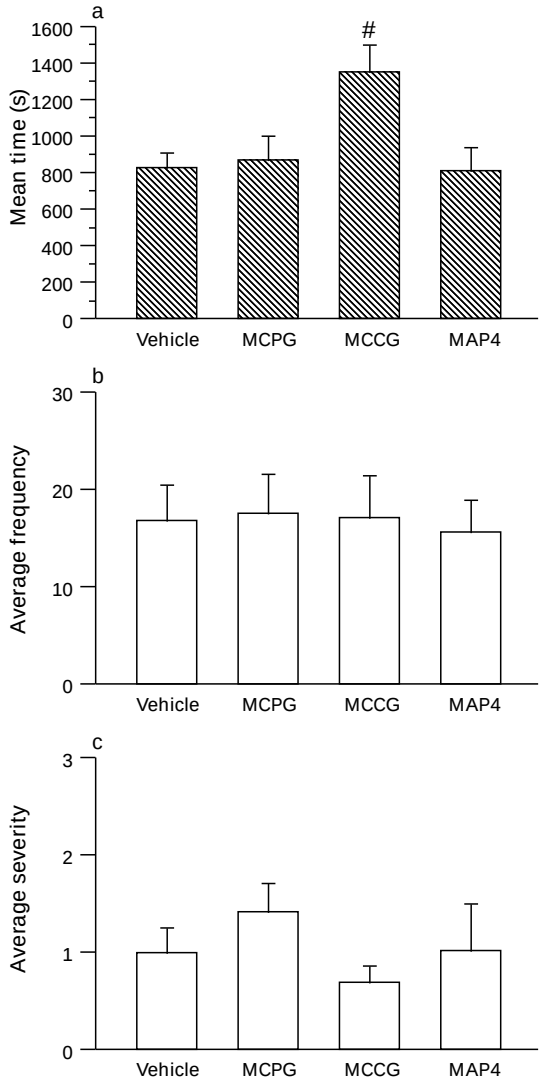

Figure 2 (a) Mean time spent in withdrawal (teeth chattering and writhing combined) during the 40 min withdrawal for morphine-dependent rats give an acute i.c.v. injection of either vehicle, or 2 nmol of MCPG, MCCG or MAP4 10 min before the precipitation of withdrawal. Vertical lines show s.e.mean #Significantly greater than vehicle (LSD t test, $P<0.05$) (b) Average frequency of counted symptoms (jumps and wet dog shakes combined) during the 40 min withdrawal period for morphine-dependent rats given an acute i.c.v. injection of either vehicle, MCPG, MCCG or MAP4 just before the precipitation of withdrawal. (c) Average severity of agitation for morphine-dependent rats given an acute i.c.v. injection of either vehicle, MCPG, MCCG or MAP4.

To verify that acute i.c.v. injection of mGluR antagonists had no significant effects on general behaviour, non-withdrawal and withdrawal behaviours were compared during the 10 min before i.c.v. injection, the 10 min after i.c.v. injection but before naloxone, and the 10 min after naloxone injection in non-dependent and morphine-dependent rats given an acute i.c.v. injection of either vehicle or 2 nmol of MCPG, MCCG or MAP4. Statistics confirmed that rats were more active in earlier time blocks (exhibiting more ambulating, rearing and grooming, and less resting) (LSD t test, $P<0.05$), and that after the injection of naloxone, morphine-dependent rats exhibited significantly more time in withdrawal and therefore less time in non-withdrawal behaviours than non-dependent rats (LSD t test, $P<0.05$). The only difference between vehicle-treated and mGluR antagonist-treated rats was that rats given MCCG exhibited significantly more time in withdrawal than vehicle-treated rats after the injection of naloxone ($P<0.05$, LSD t test) (data not shown).

Discussion

In the present study we have shown that chronic antagonism of mGluRs in general, as well as chronic antagonism of group II or III mGluRs, attenuates the severity of the precipitated morphine withdrawal syndrome. Non-selective antagonism of mGluRs with MCPG decreased the frequency of jumps and wet dog shakes, as well as the severity of agitation. Selective chronic antagonism of $mGluR_2$ and $mGluR_3$ with MCCG significantly decreased the time spent teeth chattering and writhing, the frequency of jumps and wet dog shakes, and the severity of agitation. Selective chronic antagonism of $mGluR_{4,6,7\text{ and }8}$ with MAP4 decreased the time spent teeth chattering and writhing. Although there was also a trend for MAP4 to decrease the frequency of jumps and wet dog shakes, this effect failed to reach significance. Acute i.c.v. administration of mGluR antagonists just before the precipitation of withdrawal failed to decrease the severity of abstinence symptoms, and in the case of MCCG, it actually increased the severity of the withdrawal syndrome.

Despite the fact that chronic administration of each antagonist reduced precipitated withdrawal symptoms, we did not observe a clear dose-response relationship for any of the agents. Perhaps this is due to the fact that our dose range was relatively small and the doses may have been in the same effective range. However, we avoided higher doses to prevent non-selective effects. MCPG has been shown to antagonize selectively group I and II mGluRs, with no effects on ionotropic glutamate receptors at concentrations up to 1 mM *in vitro* (Thomsen *et al*., 1994). It has been shown that MCCG is selective for group II mGluRs and MAP4 is selective for group III mGluRs at concentrations up to 300 μM *in vitro* (Jane *et al*., 1994), and had no effect on ionotropic receptors at concentrations as high as 500 μM (Jane *et al*., 1994). Although our highest infusion (40 nmol day^{-1}) is a higher concentration (1667 μM), our two lower doses (1.6 and 8 nmol day^{-1}) are within the selective concentration range (66.67 and 333.3 μM, respectively). Therefore, because the effects were evident at these lower doses, they are probably due to actions at mGluRs. There were also differences in the degree to which each antagonist affected the various behaviours. This could possibly be due to inter-subject variability, or possibly because some minor or recessive withdrawal symptoms will sometimes increase as major or dominant symptoms decrease.

While chronic i.c.v. antagonism of mGluRs effectively decreased the severity of the withdrawal syndrome, there were few effects on non-withdrawal behaviours. The only effect due to i.c.v. treatment was that antagonist-treated rats were somewhat more active than vehicle-treated rats. However, the most significant changes in general behaviours were completely independent of treatment given. Thus, rats, including those treated only with vehicle, were generally less

active later in the test session because by this time they had explored the test box and were habituated to the environment.

The ability of chronic i.c.v. administration of MCCG and MAP4 to decrease the severity of morphine withdrawal suggests a role for mGluR-regulated cyclic AMP production in the development of opioid dependence. MCCG selectively antagonizes group II mGluRs ($mGluR_2$ and $mGluR_3$), and MAP4 selectively antagonizes group III mGluRs ($mGluR_{4,6,7 \text{ and } 8}$), both of which are negatively coupled to cyclic AMP production. There is a high expression of mRNA for group II and III mGluRs, as well as opioid receptors, in thalamus, striatum and cortex (Masu *et al*., 1994; Mansour *et al*., 1995). Because activity at opioid receptors also effects cyclic AMP production, it can be hypothesized that group II and III mGluRs interact with opioid receptors via actions on cyclic AMP production. It is generally accepted that chronic opioid treatment leads to compensatory changes in cyclic AMP in neuronal tissues. Thus, whereas acute administration of μ- and δ-opioids decreases cyclic AMP production, during chronic treatment cyclic AMP production returns to near control levels, and is greatly enhanced during withdrawal (Childers, 1991). We propose that by antagonizing group II or III mGluRs, we are removing one source by which the production of cyclic AMP is decreased, thereby modulating the chronic effects of opioids and possibly eliminating the need for the elicitation of compensatory mechanisms.

As well as antagonizing group II mGluRs, MCPG also antagonizes group I mGluRs ($mGluR_1$ and $mGluR_5$) which are positively coupled to PI hydrolysis. In a previous study (Fundytus & Coderre, 1994), we showed that selective antagonism of group I mGluRs with (*S*)-4C-PG attenuated the severity of morphine withdrawal. Because activity at opioid receptors also affects PI hydrolysis, these results suggest that mGluR-mediated changes in PI hydrolysis are also involved in the development of opioid dependence. There is a high level of expression of mRNA for opioid receptors, as well as group I mGluRs, in striatum and cortex (Masu *et al*., 1994; Mansour *et al*., 1995), suggesting that group I mGluRs and opioid receptors may interact. There is evidence that while acute administration of μ-opioids decreases PI hydrolysis, during chronic administration PI hydrolysis may increase to near control levels, and during withdrawal it is greatly enhanced (Dixon *et al*., 1990; Barg *et al*., 1994; Narita *et al*., 1994; Busquets *et al*., 1995), suggesting that compensatory mechanisms may be elicited during chronic opioid treatment. We propose that by chronically antagonizing group I mGluRs, we may be decreasing PI hydrolysis and thereby counteracting compensatory increases which may be elicited by chronic opioid treatment.

In summary, we have demonstrated the involvement of group II and III mGluRs, in addition to our previous evidence for a role of group I mGluRs, in the development of opioid dependence. Therefore, treatments which target mGluRs may be valuable tools in decreasing the incidence of opioid dependence.

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