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Chronic inhibition of intracellular Ca^{2+} release or protein kinase C activation significantly reduces the development of morphine dependence

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Abstract

We have previously shown that chronic antagonism of metabotropic glutamate receptors in the brain attenuates naloxone-precipitated withdrawal symptoms in rats treated chronically with subcutaneous (s.c.) morphine. Several subtypes of metabotropic glutamate receptors are directly linked, through a guanine nucleotide regulatory protein, to the phosphatidylinositol (PI) second messenger system. In the present investigation, we assessed the effect of inhibiting the products of PI hydrolysis on the development of opioid dependence. Thus, concurrently with subcutaneous morphine, we infused intracerebroventricularly (i.c.v.) in rats, various doses of chelerythrine, which selectively inhibits the activation of protein kinase C, and thapsigargin, which inhibits the release of intracellular Ca^{2+} when given chronically. Both chelerythrine and thapsigargin reduced the severity of naloxone-precipitated abstinence symptoms when infused i.c.v. at a dose of 10 nmol/day. A single injection of either chelerythrine or thapsigargin immediately prior to the precipitation of withdrawal failed to decrease the severity of abstinence symptoms. Our results suggest that by chronically inhibiting activity of the phosphatidylinositol system, the development of morphine dependence can be attenuated.

Keywords: Opioid withdrawal; Metabotropic glutamate receptor; Protein kinase C; Inositol-1,4,5-trisphosphate; Chelerythrine; Thapsigargin

1. Introduction

Chronic treatment with opioid analgesics such as morphine leads to the development of tolerance and dependence. Tolerance is a decreased sensitivity to the effects of the drug, leading to the requirement for a higher dose to achieve the desired analgesic effect. Dependence is a continued need for the drug, following repeated administration, to maintain a state of physiological equilibrium, and results in an aversive withdrawal syndrome upon removal of the drug. Several investigators have shown that concurrent treatment with NMDA receptor antagonists attenuates the development of both tolerance to and dependence upon morphine (Marek et al., 1991a, b; Trujillo and Akil, 1991). NMDA receptors are activated by the excitatory amino acid glutamate, which also acts at two other types of ionotropic receptors (AMPA and kainate) and at metabotropic glutamate receptors (mGlu receptors) (Mayer and Westbrook, 1987; Monaghan et al., 1989).

We have previously shown that chronic antagonism of not only NMDA receptors, but also mGlu receptors, in the brain attenuates naloxone-precipitated withdrawal symptoms in rats given chronic subcutaneous morphine (Fundytus and Coderre, 1994). Metabotropic glutamate receptors are coupled directly to the cell membrane by a guanine nucleotide regulatory (G) protein (Sladeczek et al., 1985; Sugiyama et al., 1987). Activation of several subtypes of the mGlu receptors ($mGlu_{1\alpha}$, $mGlu_{1\beta}$ and $mGlu_5$) stimulates a phospholipase C catalyzed phosphatidylinositol (PI) hydrolysis (Schoepp and Conn, 1993). Phosphatidylinositol-4,5-bisphosphate (PIP_2) is hydrolyzed to produce diacylglycerol and inositol-1,4,5-trisphosphate (IP_3). IP_3 promotes the release of Ca^{2+} from internal

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stores on the endoplasmic reticulum. Diacylglycerol promotes the translocation and activation of protein kinase C (Kapcala et al., 1992).

Both acute and chronic administration of opioids has been shown to affect PI hydrolysis. Although there are some conflicting results (Smart et al., 1994), many investigators find that acute administration of selective μ-opioid receptor agonists leads to a decrease in PI hydrolysis (Barg et al., 1992, 1994; Johnson et al., 1994). With chronic administration of opioids, PI hydrolysis or protein kinase C activity has been found to be either decreased (Busquets et al., 1995; Pellegrini-Giampietro et al., 1988), unchanged (Dixon et al., 1990), or increased (Narita et al., 1994b) compared to untreated controls. However, during opioid withdrawal, PI hydrolysis or protein kinase C activity has been found to be greatly enhanced (Busquets et al., 1995; Mao et al., 1995; Pellegrini-Giampietro et al., 1988). There is also evidence suggesting that administration of opioids can affect glutamate receptor-mediated activity in a manner similar to the products of PI hydrolysis. It has been shown that application of opioids can mimic the effects of protein kinase C by enhancing glutamate-activated currents (Chen and Huang, 1991).

In addition to application of opioids affecting PI hydrolysis, it has also been demonstrated that changes in PI hydrolysis and protein kinase C activity can affect both endogenous and exogenous opioid activity. Protein kinase C interacts with endogenous opioids by stimulating secretion of both β-endorphin and its precursor, pro-opiomelanocortin (Abou-Samira et al., 1987; Kapcala et al., 1992). Moreover, activity of the PI system affects the analgesic efficacy of opioid drugs (Raffa et al., 1992; Raffa and Martinez, 1992; Zhang et al., 1990).

The present study was performed to assess whether selective chronic inhibition of PI-stimulated Ca_i^{2+} release or protein kinase C activation in the brain could attenuate the severity of the precipitated morphine withdrawal syndrome in rats. This was assessed by chronically infusing specific inhibitors of these intracellular effects intracerebroventricularly (i.c.v.). Thapsigargin initially stimulates release of Ca_i^{2+}, but prevents re-uptake into the endoplasmic reticulum, thereby inhibiting further Ca_i^{2+} release when administered chronically (Thastrup et al., 1990). Chelerythrine selectively inhibits the activation of protein kinase C by interacting directly with its catalytic domain (Herbert et al., 1990). Both thapsigargin and chelerythrine have been shown to be both cell permeable (Thastrup et al., 1990; Herbert et al., 1990) and active in vivo (Marks et al., 1991; Perchellet et al., 1993; Young et al., 1994). We also assessed the effects of a single i.c.v. injection of chelerythrine and thapsigargin immediately prior to the precipitation of withdrawal. The present study shows that chronic, but not acute, inhibition of either Ca_i^{2+} release or activation of protein kinase C attenuates the precipitated withdrawal syndrome in rats treated chronically with subcutaneous morphine.

2. Materials and methods

2.1. Subjects and surgery

Subjects were male Long Evans rats (280–350 g; Charles River, PQ, Canada). The rats were housed 2 to 4 per cage, on a 12:12 h light:dark cycle (lights on at 06:00), with food and water available ad libitum.

On Day 0 rats were anaethestized with sodium pentobarbital (Somnotol, MTC Pharmaceuticals, 60 mg/kg), and a 23 gauge stainless steel cannula was implanted stereotaxically in the lateral ventrical of each rat (AP = −1.3 mm and L = −1.8 mm from bregma, and V = −3.8 mm from the top of the skull; Paxinos and Watson, 1986). For rats given vehicle, chelerythrine or thapsigargin chronically, the cannula was attached to a Model 2001 Alzet osmotic mini pump filled with either chelerythrine, thapsigargin or vehicle (10% DMSO in saline). While the rats were still under pentobarbital anaesthesia, one unprimed (i.e. not yet pumping) Model 2ML1 Alzet pump containing 60 mg/ml morphine sulfate solution was implanted subcutaneously (s.c.) on the back of each rat. Infusion of morphine began approximately 2–4 h following implantation. On the following day, Day 1, rats were briefly anaesthetized with halothane and a second unprimed Model 2ML1 pump containing 60 mg/ml morphine sulfate solution was implanted s.c. on the back of each rat. This 2 day pump implantation procedure was used to reduce the risk of mortality due to the accumulation of lethal systemic morphine concentrations prior to any tolerance development. To assess the effects of chronic inhibition of intracellular messengers on behaviour in rats not dependent on morphine, some rats were given i.c.v. vehicle or 10 nmol/day of either thapsigargin or chelerythrine without concurrent morphine treatment (non-dependent). The effects of acute administration of chelerythrine and thapsigargin were assessed by observing the behaviour of non-dependent rats after a single injection of either 1 nmol chelerythrine or 0.5 nmol thapsigargin.

2.2. Drugs

Chelerythrine (Calbiochem, San Diego, CA, USA) and thapsigargin (Research Biochemicals, Natick, MA, USA) were continuously infused i.c.v. at a rate of 1 μl/h in the following doses: 0.1, 1 or 10 nmol/day. Acute injections were given i.c.v. 10 min prior to the precipitation of withdrawal in doses of 0.1 or 1 nmol chelerythrine, or 0.05 or 0.5 nmol thapsigargin. These doses of chelerythrine and thapsigargin were chosen to approximate the level received over a 1–2 h period in chronically treated rats; higher doses were found to produce side effects such as seizures. Morphine sulfate (Sabex, Montreal, PQ, USA) was continuously delivered s.c. at a rate of 10 μl/h, for a total dose of 36.65 μmol/day (28.8 mg/day).

2.3. Withdrawal measurement

Precipitated abstinence symptoms were assessed on the 7th day of morphine treatment after injection of the opioid antagonist naloxone (1 mg/kg, s.c.). Rats given vehicle, chelerythrine or thapsigargin chronically were observed for 10 min before and 40 min after naloxone injection. 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. The time spent in non-withdrawal behaviours (ambulating, rearing, grooming and resting) was also measured. The time spent in withdrawal and non-withdrawal behaviours was also measured for comparison, for 10 min before and after the injection of naloxone, in non-dependent rats (not given morphine) infused chronically with either vehicle or 10 nmol/day chelerythrine or thapsigargin. Rats given an acute i.c.v. injection of vehicle, chelerythrine or thapsigargin were observed for 10 min prior to i.c.v. injection, then another 10 min after i.c.v. injection but before naloxone injection, and for 40 min following naloxone injection, during which time withdrawal symptoms were assessed. In rats given an acute i.c.v. injection of vehicle, 1 nmol chelerythrine or 0.5 nmol thapsigargin, non-withdrawal and withdrawal behaviours were compared during the 10 min prior to i.c.v. injection, the 10 min after i.c.v. injection but before naloxone injection, and 10 min after naloxone injection in both non-dependent and morphine-dependent rats.

2.4. Statistical analysis

Timed withdrawal behaviours (teeth chattering, writhing) were analyzed using planned comparisons between experimental groups and the vehicle control group. Counted withdrawal behaviours (number of jumps and wet dog shakes) were analyzed using a Kruskal-Wallis ANOVA for non-parametric data, followed by Mann-Whitney U-tests on significant main effects.

The effect of chronic inhibition of Ca_i^{2+} release and protein kinase C activation on non-withdrawal behaviours (ambulating, rearing, grooming and resting) was assessed by comparing the first two time blocks (i.e. 10 min prior to naloxone injection and 10 min after naloxone injection) for rats in each treatment group. The effects of acute i.c.v. injection of chelerythrine and thapsigargin was assessed by comparing non-withdrawal and withdrawal behaviours for the first three time blocks (i.e. 10 min before i.c.v. injection, 10 min after i.c.v. but before naloxone injection, and 10 min after naloxone injection). 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 percentage of time spent in each behaviour. Significant effects were further assessed with Tukey's post-hoc tests for samples of unequal sizes.

3. Results

Fig. 1 illustrates the severity of abstinence symptoms observed during the 40 min withdrawal period for morphine-dependent rats treated chronically with i.c.v. vehicle, chelerythrine or thapsigargin. Chronic s.c. administration of 36.65 μmol/day of morphine sulfate produced an intense and reliable withdrawal syndrome, evidenced by

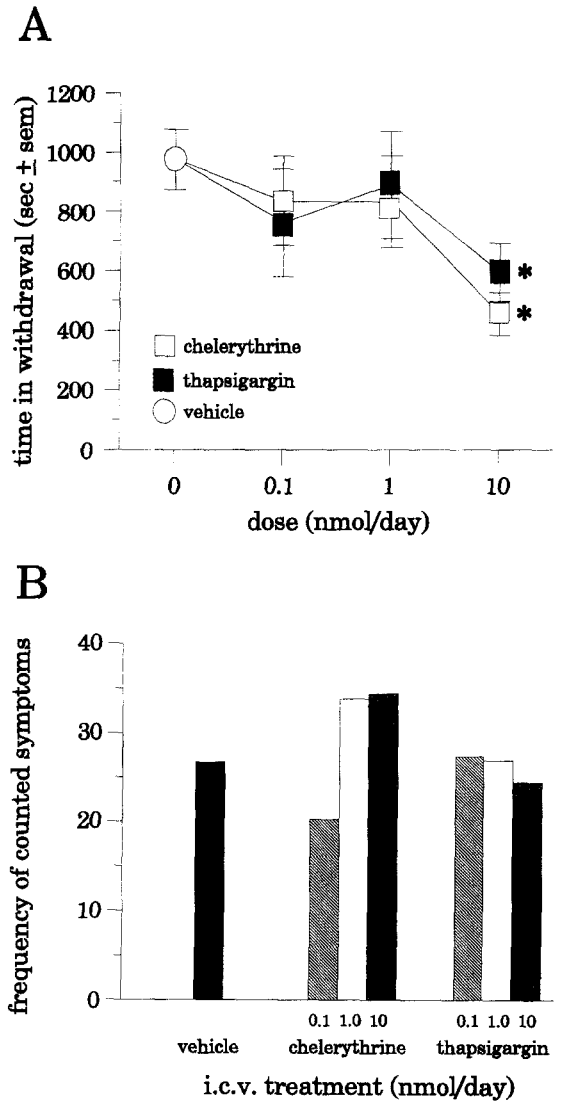

Fig. 1. (A) Mean time spent exhibiting teeth chattering and writhing (time in withdrawal) during the 40 min withdrawal period for morphine-dependent rats treated chronically with either vehicle, chelerythrine or thapsigargin i.c.v ($n = 4$–8 per group). There appears to be a dose-related inhibition of withdrawal symptoms by both chelerythrine and thapsigargin. Planned comparisons indicated that 10 nmol/day of either chelerythrine ($F(1,30) = 8.64$, $P < 0.01$) or thapsigargin ($F(1,31) = 5.32$, $P < 0.05$) significantly decreased the time spent in withdrawal. * Significantly different from control, $P < 0.05$, planned comparison. (B) Frequency of counted symptoms during the 40 min withdrawal period for morphine-dependent rats treated chronically with either vehicle, chelerythrine or thapsigargin i.c.v. ($n = 4$–8 per group). Kruskal-Wallis test for non-parametric data showed no significant effects of either chelerythrine ($H(3,23) = 3.57$, $P > 0.05$) or thapsigargin ($H(3,23) = 0.52$, $P > 0.05$).

the occurrence of teeth chattering, writhing, jumping and wet dog shaking.

Fig. 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 vehicle, or 0.1, 1 or 10 nmol/day thapsigargin or chelerythrine i.c.v. Both chelerythrine and thapsigargin produced a reduction in the amount of time spent in withdrawal. The highest dose of each agent, 10 nmol/day, significantly decreased the amount of time spent in withdrawal.

Fig. 1B shows the frequency of counted symptoms (jumps and wet dog shakes combined) during the 40 min withdrawal period for morphine-dependent rats in each chronic i.c.v. treatment group. Although chelerythrine appears to slightly increase the number of jumps and wet dog

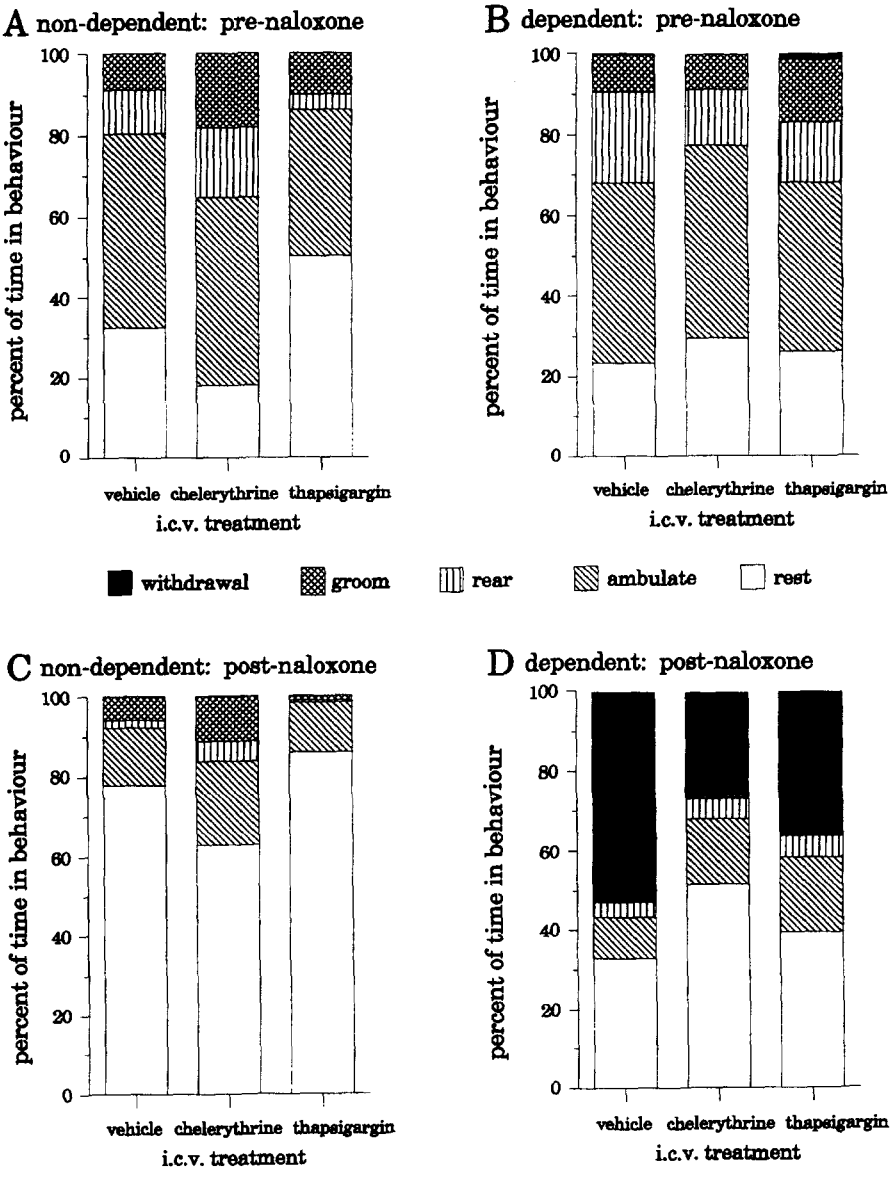

Fig. 2. Percentage of time spent in non-withdrawal (ambulating, rearing, resting and grooming) and withdrawal behaviours (teeth chattering and writhing combined) for rats treated chronically with either i.c.v. 10% DMSO, or 10 nmol/day of chelerythrine or thapsigargin i.c.v. alone (non-dependent; A) or with s.c. morphine (morphine-dependent; B) during the 10 min *prior* to the injection of naloxone (1 mg/kg, s.c.); and in rats treated chronically with i.c.v. vehicle, chelerythrine or thapsigargin either alone (non-dependent; C) or with s.c. morphine (morphine-dependent; D) during the 10 min *after* the injection of naloxone ($n = 4$–8 per group). ANOVA indicated a significant i.c.v. treatment by morphine treatment interaction ($F(2,31) = 5.08$, $P < 0.05$) and a significant morphine treatment by time interaction ($F(2,31) = 14.50$, $P < 0.01$) for the percentage of time spent resting. There was a significant time block effect for the percentage of time spent ambulating ($F(1,31) = 222.80$, $P < 0.01$). The percentage of time spent grooming also showed a significant effect of time block ($F(1,31) = 34.79$, $P < 0.01$). ANOVA indicated a significant effect of morphine treatment ($F(1,31) = 4.99$, $P < 0.05$) and time ($F(1,31) = 53.32$, $P < 0.01$) for the percentage of time spent rearing. For the percentage of time spent in withdrawal there was a significant i.c.v. treatment by morphine treatment by time interaction ($F(2,31) = 3.97$, $P < 0.05$). See Results for a description of specific differences indicated by Tukey's post-hoc tests.

shakes, there were no significant differences in the frequency of counted symptoms between any of the treatment groups.

Fig. 2 illustrates the percentage of time spent in non-withdrawal (ambulating, rearing, grooming and resting) and withdrawal (teeth chattering and writhing) behaviours for both non-dependent and morphine-dependent rats treated chronically with i.c.v. vehicle, or 10 nmol/day of either chelerythrine or thapsigargin i.c.v., during the 10 min prior to naloxone injection (A and B), and during the 10 min after naloxone injection (C and D). Statistics confirmed that, regardless of i.c.v. treatment, rats were more active prior to the injection of naloxone, when they spent more time ambulating, rearing and grooming, than after naloxone, when they spent more time resting. Morphine-dependent rats were generally less active than non-dependent rats, and as expected showed more withdrawal behaviours after the injection of naloxone. Specific comparisons at each time period showed that there were no differences between i.c.v. treatment groups prior to the injection of naloxone for either non-dependent or morphine-dependent rats, or for non-dependent rats after the injection of naloxone ($P > 0.05$). After the injection of naloxone, morphine-dependent rats given chelerythrine or thapsigargin spent more time in non-withdrawal behaviours ($P < 0.05$) and less time in withdrawal than morphine-dependent rats given vehicle ($P < 0.05$; C and D).

Fig. 3 illustrates the severity of abstinence symptoms during the 40 min withdrawal period for rats treated chronically with s.c. morphine, and given a single i.c.v. injection of vehicle, chelerythrine or thapsigargin 10 min prior to the precipitation of withdrawal. As before, chronic s.c. morphine sulfate (36.65 μmol/day) produced an intense and reliable abstinence syndrome evidenced by the occurrence of teeth chattering, writhing, jumping and wet dog shaking.

Fig. 3A shows the amount of time spent in withdrawal (teeth chattering and writhing combined) during the 40 min withdrawal period for morphine-dependent rats given a single i.c.v. injection of either vehicle, 0.1 or 1 nmol chelerythrine, or 0.05 or 0.5 nmol thapsigargin. Although 1 nmol of chelerythrine appeared to decrease the amount of time spent in withdrawal, the results failed to reach significance.

Fig. 3B shows the frequency of counted symptoms (jumps and wet dog shakes combined) during the 40 min withdrawal period for morphine-dependent rats given a single i.c.v. injection of vehicle, chelerythrine or thapsigargin. The frequency of counted symptoms was significantly increased by 1 nmol of chelerythrine.

Fig. 4 illustrates the percentage of time spent in non-withdrawal and withdrawal behaviours for both non-dependent and morphine-dependent rats given a single i.c.v. injection of either vehicle, 1 nmol chelerythrine or 0.5 nmol thapsigargin, during the 10 min prior to i.c.v. injection (A and B), the 10 min after i.c.v. injection but before naloxone injection (C and D), and the 10 min after naloxone injection (E and F). Statistics confirmed that, again, regardless of i.c.v. treatment, rats were more active earlier in the test session, with more time spent ambulating,

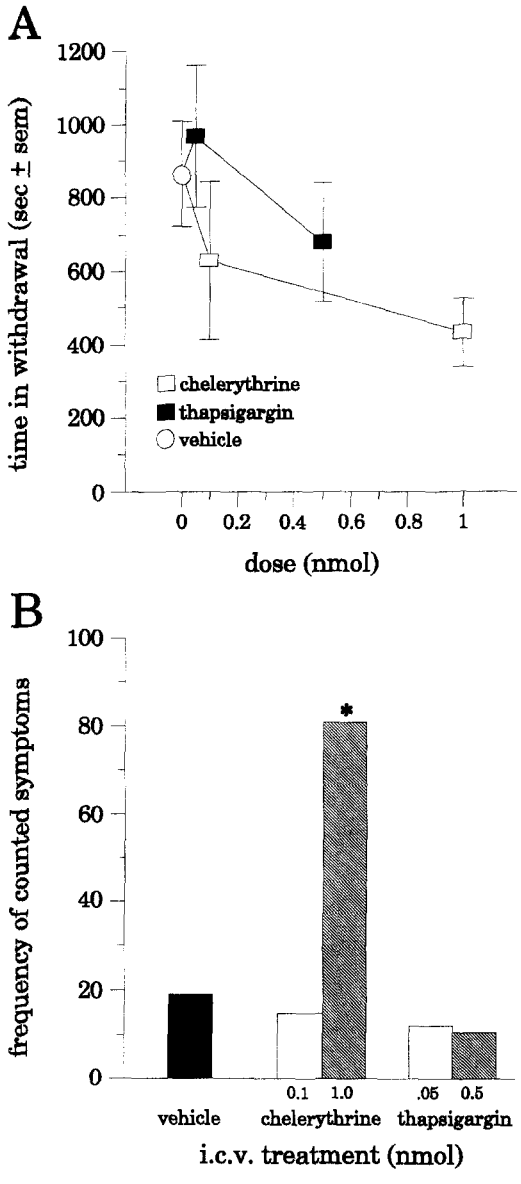

Fig. 3. (A) Mean time spent exhibiting teeth chattering and writhing (time in withdrawal) during the 40 min withdrawal for morphine-dependent rats given a single i.c.v. injection of either vehicle, or 0.1 or 1 nmol chelerythrine, or 0.05 or 0.5 nmol thapsigargin 10 min prior to precipitation of withdrawal ($n = 4–8$ per group). Although 1 nmol of chelerythrine appeared to decrease the time spent in withdrawal, the results failed to reach significance (planned comparison $F(1,13) = 3.60$, $P = 0.08$). (B) Frequency of counted symptoms during the 40 min withdrawal period for morphine-dependent rats given a single i.c.v. injection of vehicle, chelerythrine or thapsigargin ($n = 4–8$ per group). Kruskal-Wallis ANOVA for non-parametric indicated a significant effect of chelerythrine ($H(2,16) = 7.31$, $P < 0.05$). Post-hoc Mann-Whitney U-tests indicated that 1 nmol chelerythrine significantly increased the frequency of counted symptoms ($P < 0.05$). * Significantly different from control, $P < 0.05$, Mann-Whitney U-test.

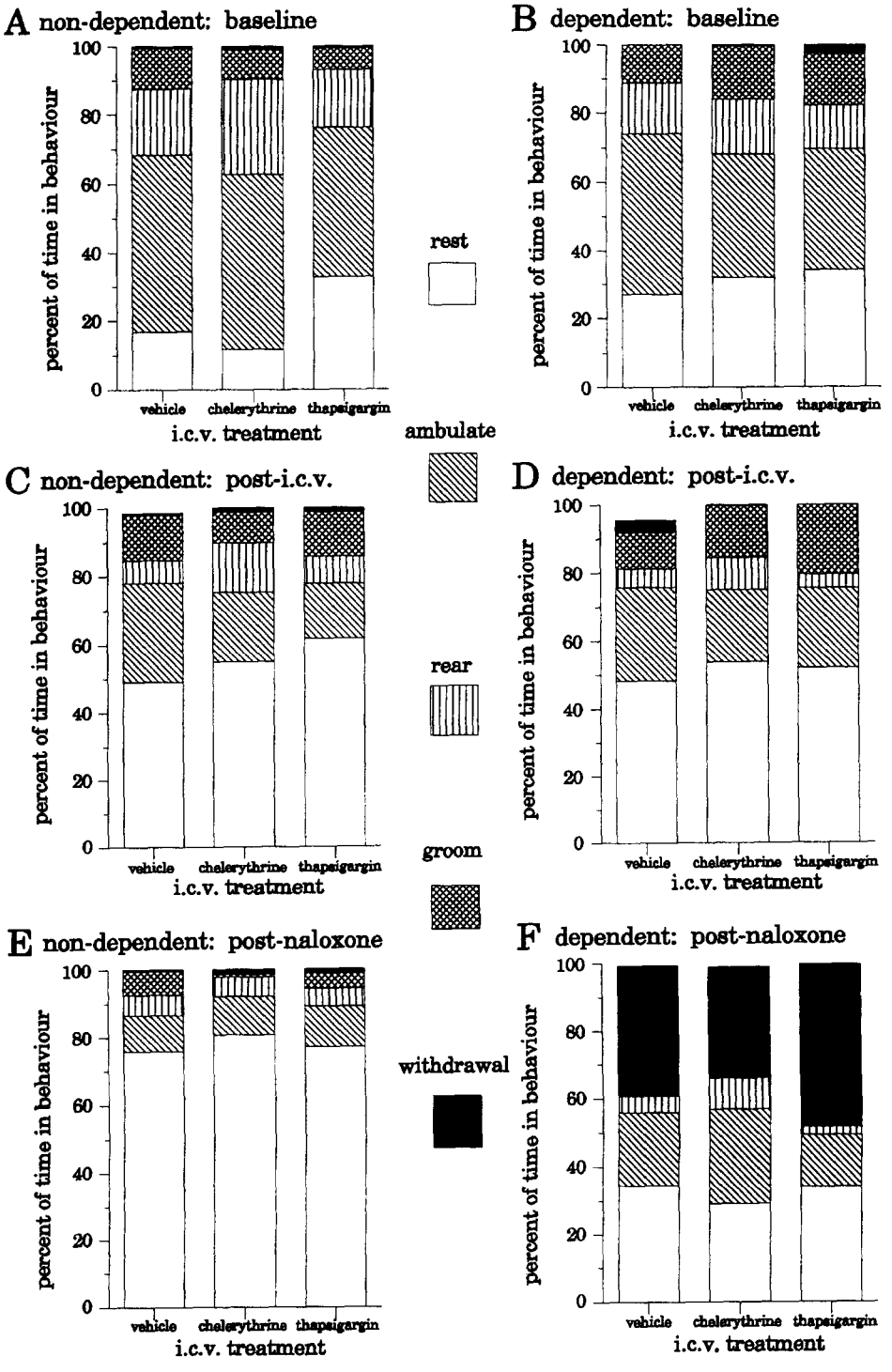

Fig. 4. Percentage of time spent in non-withdrawal (ambulating, rearing, resting and grooming) and withdrawal (teeth chattering and writhing combined) behaviours for rats given a single i.c.v. injection of vehicle, 1 nmol chelerythrine or 0.5 nmol thapsigargin alone (non-dependent; A) or with s.c. morphine (dependent; B) during the 10 min *prior* to i.c.v. injection; in rats given a single i.c.v. injection of vehicle, chelerythrine or thapsigargin alone (non-dependent; C) or with s.c. morphine (dependent; D) during the 10 min *after* i.c.v. injection but *prior* to naloxone injection; and in non-dependent (E) and morphine-dependent (F) rats given a single i.c.v. injection during the 10 min *after* the injection of naloxone (1 mg/kg, s.c.). ANOVA indicated a significant morphine treatment by time interaction for the percentage of time spent resting ($F(2,58) = 31.19$, $P < 0.01$). For the percentage of time spent ambulating, ANOVA indicated a significant i.c.v. effect ($F(2,29) = 5.01$, $P < 0.05$) and a significant morphine treatment by time interaction ($F(2,58) = 13.23$, $P < 0.01$). There was a significant time effect for the percentage of time spent grooming ($F(2,58) = 17.39$, $P < 0.01$). ANOVA indicated a significant morphine treatment by time interaction for the percentage of time spent rearing ($F(2,58) = 4.10$, $P < 0.05$). ANOVA indicated a significant morphine treatment by time interaction for the percentage of time spent in withdrawal ($F(2,58) = 59.27$, $P < 0.01$). See Results for a description of specific differences as indicated by Tukey's post-hoc tests.

rearing and grooming and less time spent resting, than later in the test session. Morphine-dependent rats were generally less active than non-dependent rats, and exhibited more withdrawal symptoms after naloxone injection. There were no differences on any behaviours between i.c.v. treatment groups, except that thapsigargin-treated rats ambulated less than vehicle-treated rats ($P < 0.05$).

4. Discussion

The present results suggest that PI hydrolysis contributes significantly to the development of dependence with chronic opioid use. Chronic inhibition of products of PI hydrolysis (IP_3-stimulated Ca_i^{2+} release or diacylglycerol-mediated protein kinase C activation) concurrently with morphine treatment significantly decreased the severity of timed withdrawal symptoms, while having very little effect on non-withdrawal behaviours. Both chelerythrine and thapsigargin, at the highest dose used, 10 nmol/day, produced a significant reduction in timed withdrawal behaviours. A single i.c.v. injection of either chelerythrine or thapsigargin was ineffective in reducing withdrawal behaviours. Although 1 nmol of chelerythrine appeared to decrease time spent in withdrawal, the results failed to reach significance. Furthermore, this dose of chelerythrine unexpectedly increased the frequency of counted symptoms.

In both acute and chronic experiments, rats spent more time ambulating, rearing and grooming early in the test session. As the test session progressed, the time spent resting increased and activity decreased, most likely because by this time they had explored the test box thoroughly and were habituated to the environment. This finding is common in behavioural observations. Furthermore, after the injection of naloxone, morphine-dependent rats in both acute and chronic experiments spent more time in withdrawal and less time in non-withdrawal behaviours than non-dependent rats, as one might expect. For both acutely and chronically treated rats, there were no differences between i.c.v. treatment groups, with a few exceptions. Rats given an acute i.c.v. injection of 0.5 nmol thapsigargin ambulated less than rats given an acute i.c.v. injection of vehicle. Chronic i.c.v. treatment with either chelerythrine or thapsigargin also decreased the time spent in withdrawal (and therefore increased the time spent in non-withdrawal behaviours) compared to chronic vehicle-treated rats after the injection of naloxone.

Recently, experimental findings suggest that there are interactive effects between opioids, glutamate and the products of PI hydrolysis (e.g. protein kinase C and IP_3). It has been demonstrated in cultures of spinal trigeminal neurons in thin medullary slices from rats that acute application of the μ-opioid agonist [D-Ala^2-MePhe^4-Gly-ol^5]enkephalin (DAMGO) potentiates glutamate-activated currents (Chen and Huang, 1991). This effect of DAMGO seemed to involve protein kinase C, as administration of protein kinase C produced similar effects, and addition of a protein kinase C inhibitor attenuated the current enhancing effects of both protein kinase C and DAMGO (Chen and Huang, 1991).

Activation of the PI system also affects endogenous opioid systems. It has recently been shown that protein kinase C activators can stimulate secretion of the endogenous opioid β-endorphin from cultured hypothalamic cells (Kapcala et al., 1992). Furthermore, the secretion of pro-opiomelanocortin, from which β-endorphin is formed, can be stimulated in the anterior pituitary by activating protein kinase C (Abou-Samira et al., 1987).

Moreover, alterations in PI hydrolysis have been demonstrated to affect opioid analgesia. Pretreatment of mice with lithium chloride reduced the antinociceptive action in the tail-flick test of several μ-opioid receptor agonists: morphine, DAMGO and sufentanil (Raffa et al., 1992; Raffa and Martinez, 1992). Lithium chloride inhibits the activity of the enzyme inositol 1-phosphatase, thereby blocking resynthesis of PIP_2 from inositol phosphate (IP) and decreasing PI hydrolysis (Li et al., 1993; Manji et al., 1993). Raffa and colleagues (Raffa et al., 1992; Raffa and Martinez, 1992) also found that treatment with IP_3, which is produced by PI hydrolysis, restored the efficacy of the opioid analgesics in mice pretreated with lithium chloride. These results suggest that opioid analgesia may be mediated at least in part by increased PI hydrolysis. However, other investigators (Zhang et al., 1990) observed that activating protein kinase C, a product of PI hydrolysis, attenuated analgesia produced by μ-, δ- and κ-opioid receptor agonists. Thus, the interaction between opioids and the PI system appears to be a complicated one.

Many investigators have measured PI hydrolysis following opioid administration. When using systems in which μ-opioid receptors are predominant, or when selective μ-opioid agonists are used, a decrease in PI hydrolysis is generally observed upon acute administration of opioid agonists (Barg et al., 1992, 1994; Johnson et al., 1994; but also see Smart et al., 1994). Conversely, in systems which are mediated predominantly by δ- or κ-opioid receptors, or when selective δ- or κ-opioid receptor agonists are used, an enhancement of PI hydrolysis is generally observed (Barg et al., 1993; Feng et al., 1994; Jin et al., 1994; Leach et al., 1986; Okajima et al., 1993; Periyasamy and Hoss, 1990; Smart et al., 1994; Tsu et al., 1995; but see also Yu and Sadee, 1986).

Recent studies have also examined the effects of chronic opioid treatment on PI hydrolysis. Hydrolysis has been measured during both the tolerant/dependent state (prior to the precipitation of withdrawal) and during the withdrawal state. There is some disagreement as to the activity of the PI system during the tolerant/dependent state. Dixon et al. (1990) found decreased PI hydrolysis, as indicated by decreased levels of IP, IP_2 (inositol bisphosphate) and IP_3, in cerebral cortices taken from rats that had

been treated continuously with morphine for 24 h, as compared to an untreated control group. They also found a decrease in the norepinephrine-stimulated accumulation of the products of PI hydrolysis. Similarly, Busquets et al. (1995) observed that in brains taken from human heroin addicts that had died of an overdose, PI hydrolysis was decreased, as indicated by a decreased level of protein kinase C-$\alpha\beta$ in frontal cortex. Moreover, these same investigators found that in dependent rats treated chronically with morphine, PI hydrolysis was decreased as indicated by decreased levels of protein kinase C-$\alpha\beta$ in frontal cortex compared to untreated controls, while in chronic morphine-treated rats undergoing naloxone-precipitated withdrawal, there were increased levels of protein kinase C-$\alpha\beta$. Conversely, Pellegrini-Giampietro et al. (1988) observed that in cortical slices taken from dependent rats (cultured in medium containing morphine) norepinephrine- and carbachol-induced PI hydrolysis, measured by levels of inositol phosphates, was not different from that observed in cortical slices taken from non-dependent rats. Under withdrawal conditions (where the morphine in the medium is replaced by naloxone) the norepinephrine- and carbachol-induced PI hydrolysis and accumulation of inositol phosphates was greatly enhanced (Pellegrini-Giampietro et al., 1988), similar to the withdrawal effects observed by Busquets et al. (1995). Narita et al. (1994b) found increased PI hydrolysis, as indicated by increased protein kinase C activity, in rats treated chronically with morphine. In these studies, PI hydrolysis after chronic opiate treatment was compared only to untreated controls, and not to rats or tissues which had been given a single (acute) opiate treatment. If acute opioid treatment produces a highly significant decrease in PI hydrolysis, then in chronic opioid-treated rats PI levels which are slightly lower or slightly greater than control values may in fact represent a compensatory increase in activity of the PI system, as seen in the studies conducted by Pellegrini-Giampietro et al. (1988) and Narita et al. (1994b). Thus, although these results are contradictory with respect to the activation state of the PI system during the tolerant/dependent state, they each suggest that there are significant effects of chronic opioid administration on PI hydrolysis. Moreover, they suggest that during chronic morphine treatment compensatory mechanisms may be induced in the central nervous system, with a resultant over-compensation during the withdrawal state.

Recently, it has been shown that inhibiting protein kinases can attenuate the development of tolerance to i.c.v. opioids. I.c.v. infusion of H-7, a non-selective protein kinase inhibitor concurrently with i.c.v. morphine or butorphanol, attenuated the development of analgesic tolerance to i.c.v. administered morphine or butorphanol (Narita et al., 1994a).

Recent evidence suggests that chronic opioid treatment may elicit compensatory changes in the spinal cord as well as the brain. Mao et al. (1995) found that although an acute intrathecal administration of morphine had no effect on the number of protein kinase Cγ immunostained neurons in the spinal cord dorsal horn of rats, chronic intrathecal morphine increased the number of immunostained neurons. Furthermore, it has previously been shown that coadministration of GM1 ganglioside with intrathecal morphine attenuates the development of tolerance and dependence (Mao et al., 1994; Mayer et al., 1995). Although GM1 ganglioside inhibits the translocation of protein kinase C, its effects are not selective. GM1 ganglioside also inhibits activation of phospholipase A_2 (Hungund et al., 1994), as well as modulating the activity of Ca^{2+} channels (Carlson et al., 1994; Bressler et al., 1994). Thus, it is not entirely clear that the effects of GM1 ganglioside in these experiments are necessarily due to its inhibition of the translocation of protein kinase C.

Our results indicate that chronically inhibiting either the IP_3-stimulated Ca^{2+} release or the activation of protein kinase C in the brain significantly reduces the development of morphine dependence. We suggest that opioid receptor activation does indeed lead to decreased PI hydrolysis, with a compensatory increase in the activity of the PI system during chronic opioid treatment. Thus, by inhibiting Ca_i^{2+} release or the activation of protein kinase C in the brain, both effects of PI hydrolysis, we were able to counteract the elicitation of a compensatory increase in activity of the PI system, thereby reducing the effects of PI hydrolysis on morphine dependence.

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