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Naloxone methiodide reverses opioid-induced respiratory depression and analgesia without withdrawal

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Abstract

Illicit opioid overdoses are a significant problem throughout the world, with most deaths being attributed to opioid-induced respiratory depression which may involve peripheral mechanisms. The current treatment for overdoses is naloxone hydrochloride, which is effective but induces significant withdrawal. We propose that selectively peripherally acting opioid receptor antagonists, such as naloxone methiodide, could reverse respiratory depression without inducing predominantly centrally mediated withdrawal. Acute administration of morphine (300 mg/kg, i.p.) was found to significantly depress respiratory rate and induce analgesia ($P < 0.0001$). Both naloxone hydrochloride and naloxone methiodide were able to reverse these effects but naloxone methiodide precipitated no significant withdrawal. Naloxone methiodide was also able to reverse opioid-induced respiratory depression ($P < 0.001$) and antinociception ($P < 0.01$) after chronic morphine administration (300 mg/kg/day for 5 days) without inducing significant withdrawal. Therefore, peripherally selective opioid receptor antagonists should be investigated as possible treatments for opioid-induced respiratory depression which do not induce adverse effects, such as withdrawal.
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Keywords: Morphine; Naloxone; Naloxone methiodide; Respiration; Withdrawal

1. Introduction

Overdose deaths due to illicit opioid use are a problem throughout the world (McGregor et al., 1998). In 1999, 960 opioid overdose deaths occurred in Australia between those aged 15 and 44 years (Trewin, 2001). Despite most of these deaths being attributed to illicit opioid overdoses, several reports have been published revealing overdoses in methadone maintenance patients, particularly during induction onto these treatment programs (Caplehorn and Drummer, 1999; Drummer et al., 1990, 1992; Williamson et al., 1997). Suggestions have also been made that in an attempt to reduce side effects and the possibility of accidental overdose, the dose of opioids administered therapeutically may be lower than that required for adequate pain control (Florez et al., 1983; McQuay, 1999; Yuan et al., 2000).

It is now widely accepted that opioid-induced respiratory depression contributes significantly to opioid-related deaths, but it is not clearly understood how opioids produce these respiratory effects (White and Irvine, 1999). Opioids are thought to act on the respiratory centres in the brain, but may also modulate the activity of central and peripheral chemoreceptors to depress neuronal activity and cause alterations in tidal volume and respiratory frequency (White and Irvine, 1999; Yeadon and Kitchen, 1989). There is the suggestion that a number of mechanisms are involved in these overdoses and it is not a simple dose-related depression of respiratory controls. This is derived from evidence that high blood opioid concentrations are not always observed in heroin overdoses and many deaths occur several hours after opioid administration, when blood concentrations of opioids are falling (Monforte, 1977; Richards et al., 1976; Zador et al., 1996).

It is possible that the respiratory effects of opioids may occur through effects at both central and peripheral sites. These peripheral sites may include chemoreceptors, j receptors, stretch receptors or peripherally located opioid receptors themselves, such as those identified in the lungs (Bhargava et al., 1997; Yeadon and Kitchen, 1989).

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The most common treatment for opioid overdose is naloxone hydrochloride, a nonselective opioid receptor antagonist that is highly lipid soluble and can rapidly diffuse across the blood brain barrier (Brown and Goldberg, 1985; Martin, 1976). Due to its action on all opioid receptor types regardless of location, naloxone hydrochloride will reverse respiratory depression but also induce withdrawal and antagonise any additional effects of opioids, such as analgesia. The induction of withdrawal and loss of analgesia are considered by illicit opioid users as unwanted effects of naloxone hydrochloride treatment and may add to their reluctance to seek treatment in overdose situations (Strang et al., 1996).

Quaternary derivatives of naloxone hydrochloride, such as naloxone methiodide, are thought not to cross the blood–brain barrier. They have been tested for their ability to block or reverse the peripheral effects of opioids. These studies have concentrated on the gastrointestinal tract and have been shown to be effective (Pol et al., 1995, 1996a,b; Yuan et al., 1996, 1997, 2000). The effect of these peripheral antagonists on respiration has been examined in a few studies but not with appropriate antagonist doses nor in a situation where normal respiration is significantly depressed by an opioid (Amin et al., 1994; Lee et al., 2000, 2001).

We hypothesise that use of a peripherally acting opioid receptor antagonist, such as naloxone methiodide, may reverse opioid-induced respiratory depression without the additional effects associated with naloxone hydrochloride administration. The two additional opioid effects examined in this study were (1) withdrawal, which is thought to be predominantly centrally mediated (Katovich et al., 1986; Rohde et al., 1997; Russell et al., 1982), and (2) analgesia, which has both central and peripheral components (Randich et al., 1991; Stein, 1993).

2. Methods

2.1. Animals

Male Swiss albino mice (30 ± 0.5 g) remained under constant environmental conditions in a 12-h light–dark cycle with food and water ad libitum. All observations were undertaken in clean 20-cm^3 Plexiglas monitoring cages, the observer was blinded to all treatments and each animal was used only once. All procedures were approved by the Adelaide University Animal Ethics Committee.

2.2. Drugs

Morphine sulphate was purchased from GlaxoSmithKline (VIC, Australia), naloxone hydrochloride was purchased from FH Faulding & Co. (SA, Australia) and naloxone methiodide was purchased from Sigma-Aldrich (NSW, Australia). The vehicle used for all drugs was saline (0.9% NaCl) and all injections were administered intraperitoneally (i.p.) in a volume of 5 ml/kg.

2.3. Acute treatment

To simulate an overdose situation, a high dose of morphine was used (300 mg/kg) to produce respiratory depression and analgesia without causing death. After obtaining baseline respiratory rates, mice were injected with morphine and monitored for 40 min. At the end of this time period, animals were then injected with naloxone hydrochloride at doses of 0.5, 1, 2 or 3 mg/kg or naloxone methiodide at concentrations of 30, 50, 70 or 100 mg/kg. Animals were then monitored for another 40 min and their nociceptive response tested on a 50 °C hot plate. Nociception testing was only conducted after the 40 min observation period to avoid the possibility of this testing affecting the respiratory and behavioural data being collected. Control animals were also injected with saline and then 3 mg/kg naloxone hydrochloride or 100 mg/kg naloxone methiodide, and monitored in the same manner.

To measure the respiratory changes in unrestrained animals, the respiratory rate of each animal was counted by an observer blinded to the treatments given. The number of breaths over a 5-s period was counted twice and averaged every 10 min. Withdrawal symptoms were recorded continuously, grouped for each 10-min period and graded using the method of Blasig et al. (1973). To test the effect of treatment on analgesia, animals were placed on a 50 °C hot plate until any jumping, paw shaking or paw licking was observed. Animals not exhibiting these responses were removed from the plate after 60 s (Suzuki et al., 1997).

2.4. Chronic treatment

To test the effect of continued use of high doses of morphine, animals were injected once daily with 300 mg/kg morphine for 5 days. On day 5, animals were given their final morphine injection and monitored. After 40 min, they were then injected with saline, naloxone hydrochloride (3 mg/kg) or naloxone methiodide (100 mg/kg). Observations were repeated as with the acute treatment.

2.5. Calculations and statistics

The respiratory rate (breaths/min) was converted to % baseline respiratory rate for each animal. The hot plate % maximum possible effect (%MPE) was calculated using the following equation: $\%MPE=[(\text{test latency} - \text{control latency})/(60 - \text{control latency})] \times 100$ (Carmody, 1995). The control latency (17 ± 1 s ($n=6$)) was determined from untreated animals. All data are presented as mean ± S.E.M., with $n=6$ in each group.

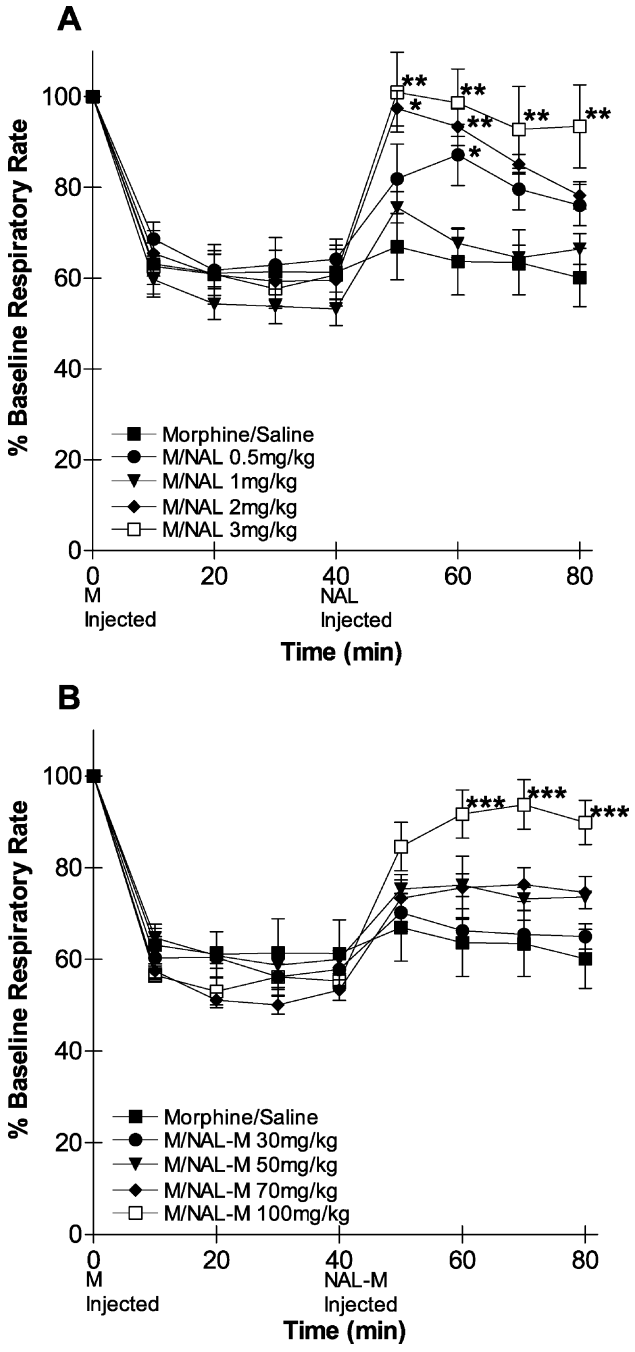

Fig. 1. (A, B) Effects of (A) naloxone hydrochloride (NAL) and (B) naloxone methiodide (NAL-M) on respiratory rate when injected 40 min after the acute administration of 300 mg/kg morphine (M). Means ± S.E.M. are shown. * $P<0.05$, ** $P<0.01$, *** $P<0.001$ compared to Morphine/Saline using one-way ANOVA with Dunnett's post hoc test.

One-way analysis of variance (ANOVA) with Dunnett's or Tukey's post hoc tests was used to determine the differences between treatment groups for antinociception and respiration over the time period observed. Withdrawal gradings were analysed using a Kruskal–Wallis test with Dunn's post hoc test. All statistical tests were performed using GraphPad Prism 3.2 and $P<0.05$ was considered statistically significant.

3. Results

3.1. Acute opioid treatment

The main aim of this study was to determine if naloxone methiodide was able to reverse the respiratory depression

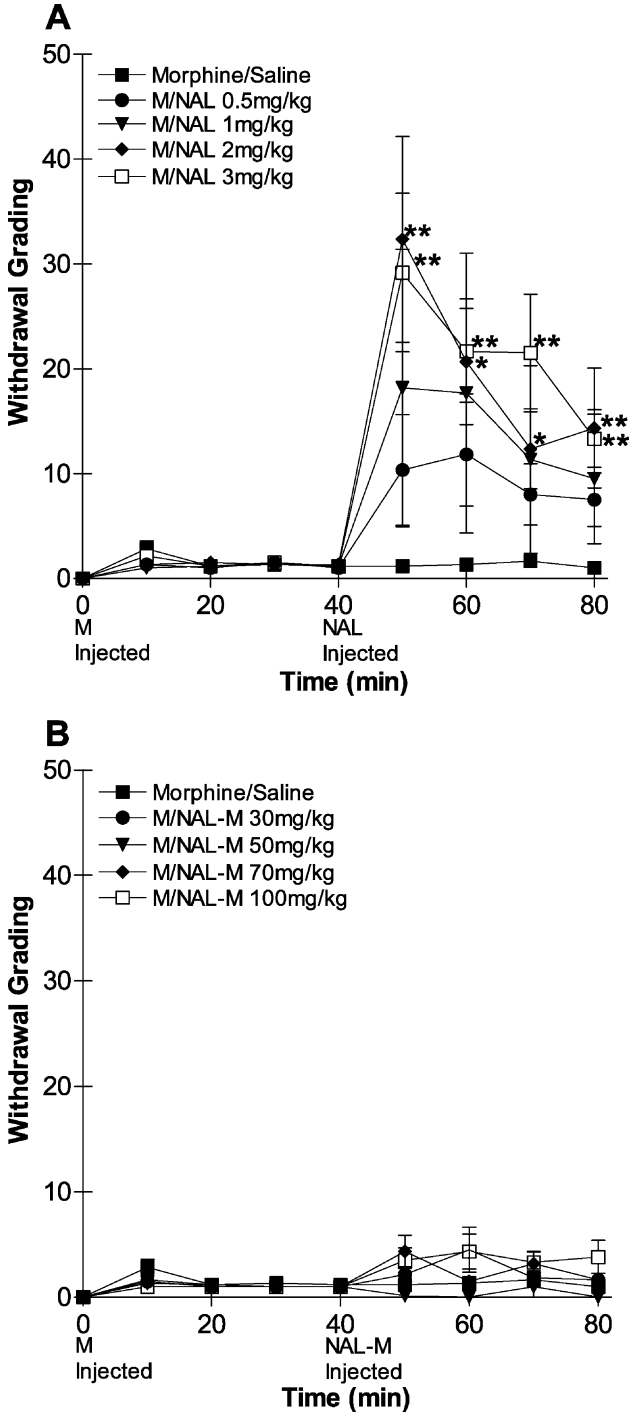

Fig. 2. (A, B) Effects of (A) naloxone hydrochloride (NAL) and (B) naloxone methiodide (NAL-M) on withdrawal gradings when injected 40 min after the acute administration of 300 mg/kg morphine (M). Means± S.E.M. are shown. * $P<0.05$, ** $P<0.01$ compared to Morphine/Saline using Kruskal–Wallis test with Dunn's post hoc test.

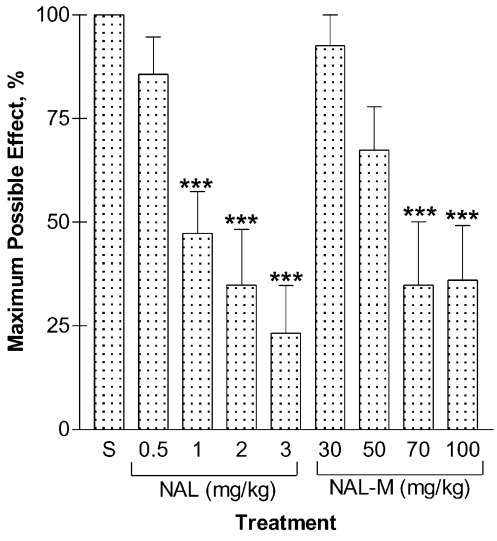

Fig. 3. Hot plate latencies 80 min after acute morphine and opioid receptor antagonist administration. Mice were injected with 300 mg/kg morphine then administered saline (S), naloxone hydrochloride (NAL) or naloxone methiodide (NAL-M) 40 min after. Means ± S.E.M. are shown. *** $P<0.001$ compared to Morphine/Saline using one-way ANOVA with Dunnett's post hoc test.

produced by morphine. 300 mg/kg morphine decreased respiration to 62.1 ± 1.3% of baseline ($P<0.0001$, $n=54$) in all animals after 10 min and remained depressed in the morphine-/saline-treated animals over the duration of the experiment (Fig. 1A and B). All animals were conscious and ambulatory after morphine administration. Only one animal died, which occurred within 15 min of the opioid being injected, suggesting this was due to an error in injection technique. A significant dose-dependent reversal of morphine effects on respiration was observed after injection of increasing doses of naloxone hydrochloride and naloxone methiodide (Fig. 1A and B). At the highest antagonist doses (naloxone hydrochloride 3 mg/kg and naloxone methiodide 100 mg/kg), respiration returned to baseline rates. The saline/antagonist control animals did not experience significant changes respiration compared to saline/saline animals, except a 13.8% decrease in respiration at 10 min for the saline-/naloxone hydrochloride-treated animals ($P<0.05$) and a 14.5% increase at 70 min in the saline-/naloxone methiodide-treated animals ($P<0.001$) (data not shown).

Naloxone hydrochloride produced a significant dose-dependent withdrawal response at both 2 and 3 mg/kg compared to saline. Naloxone methiodide, however, produced no significant withdrawal at any of the concentrations administered (Fig. 2A and B).

Morphine treatment produced maximum antinociception and a dose-dependent reversal was observed with both naloxone hydrochloride and naloxone methiodide (Fig. 3). All concentrations of naloxone hydrochloride produced a significant reduction in analgesia, while only 70 and 100 mg/kg naloxone methiodide concentrations were significantly different to morphine-/saline-treated animals. There was no difference in antinociception between the animals injected with saline, naloxone hydrochloride or naloxone methiodide in the absence of morphine (data not shown).

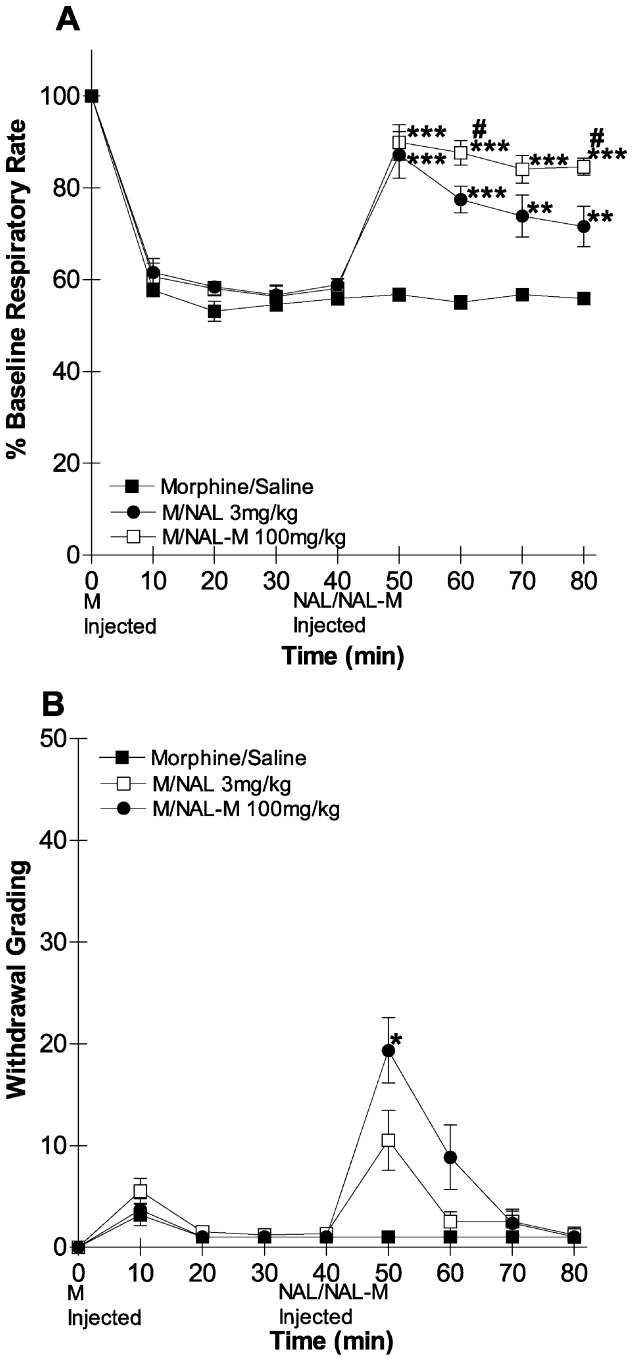

Fig. 4. (A, B) Effect of naloxone hydrochloride (NAL) and naloxone methiodide (NAL-M) on (A) respiratory rate and (B) withdrawal gradings when injected 40 min after chronic morphine (M) administration (300 mg/kg/day for 5 days). Means ± S.E.M. are shown. * $P<0.05$, ** $P<0.01$, *** $P<0.001$ compared to Morphine/Saline, # $P<0.05$ compared to Morphine/NAL 3 mg/kg using (A) one-way ANOVA with Tukey's post hoc test and (B) Kruskal–Wallis test with Dunn's post hoc test.

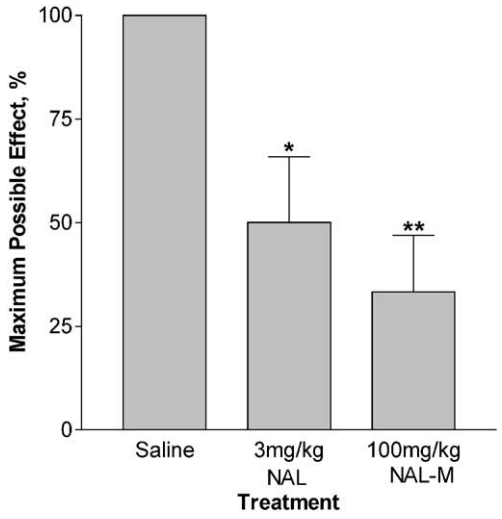

Fig. 5. Hot plate latencies 80 min after chronic morphine and acute opioid receptor antagonist administration. Mice were administered the final 300 mg/kg morphine injection then given saline, naloxone hydrochloride (NAL) or naloxone methiodide (NAL-M) 40 min after. Means ± S.E.M. are shown. * $P<0.05$, ** $P<0.01$ compared to Morphine/Saline using one-way ANOVA with Dunnett's post hoc test.

3.2. Chronic opioid treatment

Respiratory rate was decreased with the final morphine challenge on day 5 and in the morphine-/saline-treated animals remained at a depressed level during the 80 min of observation (Fig. 4). This was not significantly different to the depression observed in the acutely treated animals, or in these animals during morphine administration on day 1, so tolerance to the respiratory depressant effects of morphine did not appear to have developed during this treatment regime. Both naloxone hydrochloride and naloxone methiodide significantly increased respiration compared to the morphine-/saline-treated animals (Fig. 4A). This effect was sustained with the administration of naloxone methiodide but naloxone hydrochloride, despite producing a significant increase in respiration, did not maintain this reversal to the same extent.

The severity of withdrawal after naloxone hydrochloride challenge was less than that observed in the acute study. Despite this, the naloxone hydrochloride-treated animals still produced significant withdrawal, while the naloxone methiodide-treated animals did not (Fig. 4B). The hot plate latencies showed that both naloxone hydrochloride and naloxone methiodide produced significant reversal of opioid-induced analgesia (Fig. 5).

4. Discussion

In this study, both naloxone hydrochloride and naloxone methiodide were shown to dose dependently reverse morphine-induced respiratory depression in acutely treated mice. Significant withdrawal, however, was only produced in the naloxone hydrochloride-treated animals and not those administered naloxone methiodide.

The doses of naloxone methiodide required to produce an observable respiratory effect in this study were much higher than the doses of naloxone hydrochloride required. This is consistent with previous studies showing that naloxone methiodide has a lower potency than naloxone hydrochloride at opioid receptors (Bianchetti et al., 1982, 1983; Killian et al., 1981; Valentino et al., 1981, 1983). This means that the lack of effects observed with the systemic administration of naloxone methiodide in many previous studies may actually be a result of inadequate doses being administered (Brown and Holtzman, 1981; Lee et al., 2000; Ramabadran et al., 1982; Weber et al., 2001; Wu et al., 1997). In order to evaluate the effectiveness of naloxone methiodide much higher concentrations of this compound compared to naloxone hydrochloride that must be used. In this study, we administered doses of naloxone methiodide up to 33 times greater than that of naloxone hydrochloride (Brown and Goldberg, 1985).

It has also been suggested that naloxone methiodide may be contaminated with small quantities of naloxone, so at these high doses, naloxone is responsible for the effects observed (Bianchi et al., 1982). If this was the case, we would have observed withdrawal in the animals treated with naloxone methiodide as a result of the naloxone contamination. This did not occur. There has also been the suggestion that naloxone methiodide is being metabolised to naloxone hydrochloride or other metabolites, which then enter the brain and produce the effects observed. This metabolism would have to occur within 10 min to produce the effects seen in this study but this cannot be discounted as no studies to date have investigated the metabolism of naloxone methiodide. However, we did not observe significant withdrawal, which would have been expected if metabolites of naloxone methiodide were entering the brain. A study by Misra et al. (1987) investigating the metabolism of naltrexone methiodide after intravenous administration in the rat observed only trace concentrations of naltrexone. We therefore believe it is unlikely that the effects of naloxone methiodide on respiration are a result of naloxone either as a contaminant or as a metabolite of naloxone methiodide.

This study adds support to the hypothesis that naloxone methiodide has predominantly peripheral actions and does not readily act on the brain. Naloxone methiodide is thought not to cross the blood–brain barrier; however this has not been conclusively proven (Brown and Goldberg, 1985). If naloxone methiodide is completely excluded from the brain, these results suggest that opioid-induced respiratory effects have a large peripheral component.

The animals administered saline then naloxone hydrochloride and naloxone methiodide produced similar results to studies using golden hamsters (Schlenker and Inamdar, 1995). Breathing frequency was shown to not change with the administration of 1 mg/kg naloxone hydrochloride but a

slight, though not significant increase, was observed with the same concentration of naloxone methiodide (Schlenker and Inamdar, 1995). Only slight alterations were observed in study, which confirms that, alone, these antagonists have very little effect on normal respiration even at high doses.

The hot plate results were also of interest because naloxone methiodide was able to antagonise morphine analgesia, presumably through peripheral mechanisms that have been previously suggested (Stein, 1995). Our results support the view that opioid-induced analgesia does have a peripherally mediated component and indicates that high doses of naloxone methiodide can reverse the analgesic effects of opioids. Other researchers have reported that naloxone methiodide has no effect on opioid-induced analgesia but have used much lower doses than those examined in this study, which may explain their lack of observable effects (Ramabadran, 1982; Ramabadran et al., 1982).

This study also examined the effect of chronic morphine administration on the effects of naloxone hydrochloride and naloxone methiodide. Few studies have examined the effects of chronic opioid administration on respiration (Kokka et al., 1965; McGilliard and Takemori, 1978; Roerig et al., 1987; van den Hoogen and Colpaert, 1986). It is interesting to note that no tolerance to the respiratory or analgesic effects of morphine were evident after pretreatment. This lack of effect on respiratory rate was also shown by van den Hoogen and Colpaert (1986) in chronically morphine-treated rats breathing concentrations of CO_2 ranging from 0 to 8%, whereas McGilliard and Takemori (1978) observed a three times increase in ID_{50} for respiratory depression in morphine-pretreated mice. This highlights an area of research that requires more investigation.

Of importance in our study is that on day 5 of morphine treatment, naloxone methiodide was able to return the respiratory rate of these animals to baseline levels as in the acute experiment. Naloxone hydrochloride was also able to reverse the respiratory depression but the effect was not as sustained as that observed in the animals treated with naloxone methiodide. No significant withdrawal was observed in the naloxone methiodide-treated animals but 3 mg/kg naloxone hydrochloride produced significant withdrawal symptoms. This latter effect was not as pronounced as that seen with acute morphine treatment, which may be due to increased opioid dependence in these animals. The hot plate latencies, as in the acute study, showed that both naloxone hydrochloride and naloxone methiodide were effective in reversing the analgesic effect of morphine. Therefore, 100 mg/kg naloxone methiodide was just as effective after acute and chronic morphine treatment, but 3 mg/kg naloxone hydrochloride did not appear to produce the same degree of effect after animals were treated chronically with morphine. These findings also raise questions as to whether peripheral opioid receptor antagonists, such as naloxone methiodide, would be more suitable for use in emergency overdoses of chronic opioid users where the dose of opioid and degree of dependence of the patient is often unknown.

This study is the first to show that naloxone methiodide is effective in reversing respiratory depression in animals treated with high doses of morphine without precipitating opioid withdrawal. This reversal occurs after the acute or chronic administration of morphine. In light of the work described in this paper and the encouraging results from researchers investigating the effects of morphine on the gastrointestinal tract (Pol et al., 1995, 1996a,b) the effects of peripheral opioid receptor antagonists should be further investigated. These peripherally acting opioid receptor antagonists may be further developed to produce compounds suitable for use to prevent or treat opioid overdoses without adverse effects such as withdrawal.

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