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Use of radiotelemetry to evaluate respiratory depression produced by chronic methadone administration

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Abstract

Illicit and therapeutic opioid administration can result in overdose due to opioid-induced respiratory depression. Research investigating the respiratory depressant effects of opioids has been limited due to difficulties associated with acquiring long-term respiratory data. This study examined the novel use of radiotelemetry to measure respiratory rate, heart rate, locomotor activity and blood pressure in rats treated chronically with methadone. Over 4 days of treatment, respiratory rate decreased, but partial tolerance appeared to develop during active (night) periods. Decreased heart rate was observed during the night periods and tolerance appeared to develop to this effect. Activity and blood pressure did not change with treatment. The effects of naloxone hydrochloride and naloxone methiodide administration on the methadone-treated rats were also examined and both antagonists increased respiratory rate and heart rate, with only naloxone hydrochloride producing significant increases in activity. Radiotelemetry offers a means of evaluating drug effects on respiratory rate continually in ambulatory, unstressed animals.

Keywords: Methadone; Respiratory depression; Naloxone methiodide; Radiotelemetry

1. Introduction

Deaths due to opioid-induced respiratory depression can occur with both the therapeutic and illicit administration of opioids and contribute to many drug-related deaths in Australia and around the world (McGregor et al., 1998; Seal et al., 2003; Trewin, 2001). It has also been suggested that less than optimal doses of opioids may be administered for the treatment of pain in order to avoid respiratory depressant effects (McQuay, 1999; Salvato et al., 2003). Methadone is commonly used in opioid maintenance therapies and is also popular in the treatment of moderate to severe pain, but the potential for overdose exists in these patients, particularly during induction onto these programs (Cairns et al., 1996; Karch and Stephens, 2000; Williamson et al., 1997; Wolff, 2002). Fatal and non-fatal opioid overdoses also occur more often at night, but the reasons for this are largely unknown (Darke et al., 1996; McGregor et al., 2002; Wolff, 2002).

Whilst the non-selective opioid receptor antagonist, naloxone hydrochloride, provides an effective treatment for opioid overdose, it also produces unwanted effects, such as withdrawal and the reversal of analgesia. Furthermore, repeated dosing of naloxone hydrochloride may be required due to its short duration of action, particularly in situations when the type and dose of opioid administered is unknown (Lenton and Hargreaves, 2000; Martin, 1976). This highlights the need to determine the mechanisms underlying opioid-induced respiratory depression to identify targets for new and improved treatments.

Although the respiratory depressant effects of opioids are well known, the mechanisms of this depression, and in particular, how tolerance develops with chronic opioid treatment, are unclear. Early studies in humans have found that minimal tolerance develops to the respiratory effects of chronic morphine administration, as respiratory rates remained low in subjects throughout treatment (Martin and Jasinski, 1969). This was also shown in rhesus monkeys chronically administered L-α-acetylmethadol (LAAM) and morphine (Brandt and France, 2000; Paronis and Woods,

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1997). In mice, McGilliard and Takemori (1978a,b) and Roerig et al. (1987) found that chronic morphine treatment produced respiratory depression and only partial recovery from these effects after 72 h. Kokka et al. (1965) reported that with chronic morphine treatment rats developed tolerance to the changes in respiratory rate and oxygen consumption, but tidal volume only exhibited partial tolerance after 12 days of treatment. Significant tolerance to the respiratory effects of morphine was also observed in rats treated for 4 days (van den Hoogen and Colpaert, 1986).

In this study, we investigated the respiratory effects of methadone, which is often used as a long-term therapy with dosing regimes designed to achieve steady state concentrations. It also binds with low affinity to *N*-methyl-D-aspartic acid (NMDA) receptors, which may produce alterations in the development of tolerance and the effects observed compared to other opioid receptor agonists (Ebert et al., 1995; Gorman et al., 1997). The metabolism of methadone also differs as it is primarily metabolised by *N*-demethylation through cytochrome P450s to form inactive metabolites (Borg and Kreek, 1998).

Several studies have shown that respiration remains depressed in long-term methadone maintained patients and that if tolerance does develop to these respiratory effects it is not complete (Dyer et al., 1999; Gritz et al., 1975; Santiago et al., 1977). Animal studies investigating the effect of chronic methadone treatment on respiration are very limited. White and Zagon (1979) and McCormick et al. (1984) found that after 14 days of methadone treatment, rats experienced a decrease in pO_2 and pH and a slight increase in pCO_2, although not of the same magnitude as in the acutely treated rats. A similar result was also observed in pygmy goats administered methadone for 8 days (Neal and Olsen, 1980). This suggests that a certain degree of tolerance develops to the respiratory effects of methadone, but it is not complete. To date, no studies have investigated the respiratory effects of chronic methadone administration in a continuous fashion to determine the profile of changes that occurs over 24 h and over several days of treatment.

Until now, there have been several limitations to obtaining respiratory data in small animals during chronic opioid treatment regimes. The techniques required to measure respiratory rate, such as plethysmography or pneumotachography, involve moving animals from their natural environments into potentially stressful conditions, which can affect endogenous opioid systems and alter respiration (Vaccarino and Kastin, 2001). In addition, these methods do not facilitate the long-term measurement of respiratory parameters as the animals cannot remain in the required apparatus for extended periods, such as days.

In an attempt to explore an alternative method of measuring respiratory parameters, we investigated the use of radiotelemetry implants to measure respiratory rate in conscious, unrestrained rats. The radiotelemetry implants (Data Sciences International) used are capable of measuring heart rate, blood pressure (systolic, diastolic and mean arterial) and spontaneous locomotor activity. Using new RespiRATE software, a waveform of respiratory rate can also be derived from blood pressure signals by fitting a curve to beat to beat systolic points (Kramer et al., 1999). This allows for the continuous (every min) measurement of respiratory and cardiovascular parameters of animals in their normal environment and without stress artefacts. Whilst only respiratory rate can be collected using this technology, previous studies have shown that respiratory rate alone is a good quantitative index of opioid-induced respiratory depression in rodents (McGilliard and Takemori, 1978b). Other respiratory measurements, such as tidal volume and minute volume, are required to gain a full picture of the respiratory effects occurring, but the advantages of this continuous, unrestrained recording method outweigh the absence of these additional respiratory parameters.

Therefore, in this novel study we sought to validate the use of radiotelemetry to measure cardiovascular and respiratory changes continuously in chronically methadone-treated rats. We also examined the effect of treatment with naloxone hydrochloride and its quaternary derivative, naloxone methiodide, which we have previously shown to effectively reverse acute and chronic morphine-induced respiratory depression in mice (Lewanowitsch and Irvine, 2002).

2. Materials and methods

Male Sprague–Dawley rats of 317 ± 7 g were housed in an undisturbed, temperature controlled environment (20 ± 2 °C) with food and water ad libitum. All animals were maintained in a 12-h light–dark cycle with lights being automatically turned on at 7:30 AM and off at 7:30 PM. After radiotelemetry implantation, rats were placed into separate cages and all animal treatments were undertaken at the same time to ensure identical conditions throughout the experiment. All procedures were approved by the University of Adelaide Animal Ethics Committee.

2.1. Implantation of radiotelemetry transmitters

The implantation procedure has been described previously (Chan et al., 1999). Briefly, rats were anaesthetised with a combination of 9:1 sodium methohexitone (10 mg/ml) and sodium pentobarbitone (60 mg/ml) in a dose volume of 5 ml/kg i.p. The radiotelemetry implants (TA11-PA-C40, Data Sciences International, USA) were inserted aseptically into the peritoneal cavity with the catheter being inserted into the descending aorta above the iliac bifurcation and fixed in place using tissue adhesive (Vetbond, 3M animal care products, USA). The body of the implant was immobilized by suturing to the abdominal wall and the abdomen closed with sutures and wound clips. Topical antibiotic powder (Apex Laboratories, Australia) was applied and antibiotic (0.2 ml Tribrissen) administered s.c. at

the end of the surgery. Antibiotic (0.1 mg/ml oxytetracycline hydrochloride) was also placed into the drinking water for the subsequent 5 days when the wound clips were also removed. The animals were allowed to recover for at least 7 days prior to experimentation.

2.2. Preparation and implantation of osmotic mini-pumps

The osmotic mini-pumps (Alzet model 2ML1, Alza, USA) used in this study hold 2.1ml of fluid and administer drugs for 7 days at a rate of 9.2 μl/h. Methadone hydrochloride (300 mg/kg) was dissolved in saline (0.9% NaCl) so that each animal received a dose of 30 mg/kg/day. The mini-pumps were incubated in saline at 37 °C for 4 h prior to surgery to ensure the methadone administration would commence immediately upon implantation.

Prior to the implantation of osmotic mini-pumps, baseline measurements were taken for 24 h. On the first day of treatment (Day 1), the animals were briefly anaesthetised with fluothane, a small subcutaneous pocket cut into the dorsum of the rat, the mini pumps inserted and the pocket closed with wound clips. The animals were immediately returned to their cages where radiotelemetry measurements were taken continuously until the end of the treatment regime.

2.3. Experimental protocol

During radiotelemetry recordings, the individual rat cages were placed above the radiotelemetry receivers (RA1010, Data Sciences International) and recordings were taken for 10 s every min. The data were collected using LabPro software equipped with the RespiRATE plug-in (Data Sciences International) that allows the measurement of respiratory rate from blood pressure traces (Kramer et al., 1999).

After the baseline recordings, animals were implanted with the methadone filled mini-pumps and recordings taken for 4 days. On treatment Days 1–4, animals were injected with saline at the same time each day to provide baseline data for the subsequent antagonist treatment days. On Days 5, 6 and 7, animals were injected with either the saline vehicle (3 ml/kg i.p.), naloxone hydrochloride (3 mg/kg i.p.) or naloxone methiodide (100 mg/kg i.p.) so that all animals randomly received each treatment. No order effects were observed. The antagonist doses administered in this experiment have previously been shown to reverse respiratory depression in mice (Lewanowitsch and Irvine, 2002). The higher doses of naloxone methiodide are required due to its lower affinity for μ, δ and κ opioid receptors compared to naloxone hydrochloride (Lewanowitsch and Irvine, 2003). The serum half-life of naloxone hydrochloride in rats is approximately 30 min and while the half-life of naloxone methiodide is not known, a similar quaternary opioid receptor antagonist, methylnaltrexone, has a half-life of 45 min (Misra et al., 1987; Yeadon and Kitchen, 1988). This means that the 24-h period between each antagonist treatment is ample time to allow for the elimination of the antagonist effects and for the parameters measured to return to pre-antagonist treatment levels.

2.4. Analysis

During methadone treatment, the data were split into day and night for each day of treatment. The averages for the day and night measurements were calculated between the hours of 10 AM and 6 PM for day and 10 PM and 6 AM for night to avoid any fluctuations that may have occurred due to the lights being switched on or off. Changes between the day and night periods on each day of treatment were analysed using a paired samples *t*-test and changes from the baseline day and night recordings were analysed using a Repeated Measures Analysis of Variance (ANOVA) with Dunnett's or Tukey's post hoc test.

The averages for each of the parameters recorded after the antagonist or saline treatments were calculated from the data collected 40 min after injection. The changes between the naloxone hydrochloride, naloxone methiodide and saline treatments were analysed using a one-way ANOVA with Tukey's post hoc test. All data presented is shown as mean ± S.E.M. ($n = 4$) with statistical significance set at $P < 0.05$. All graphical and statistical manipulation was performed using GraphPad Prism 4.0 for Windows.

2.5. Drugs

Sodium methohexital (Eli Lilly, Australia), sodium pentobarbitone (Rhone Merieux, Australia), Tribrissen (Jurox, Australia), Oxytetracycline hydrochloride (CCD Animal Health, Australia) and fluothane (Zeneca, UK) were used during the surgical procedures of this study. Methadone hydrochloride was purchased from GlaxoSmithKline (Australia), naloxone hydrochloride was purchased from Mayne Pharma (Australia) and naloxone methiodide was purchased from Sigma-Aldrich (Australia).

3. Results

In order to illustrate the continuous nature of the data collected using radiotelemetry, Fig. 1 shows the data collected over Day 4 of the methadone treatment. Data were collected every minute, but for clarity the recordings from every 10 min are shown. A clear differentiation between the active nighttime and inactive daytime periods can be made as locomotor activity, respiratory rate and heart rate all increased throughout the night. Therefore, despite chronic methadone treatment over this period day/night differences in circadian rhythms were still observed.

The averages for the day and night period over the baseline and the 4 days of chronic methadone treatment are shown in Fig. 2. At baseline, the average respiratory rate was significantly higher during the night period than the day

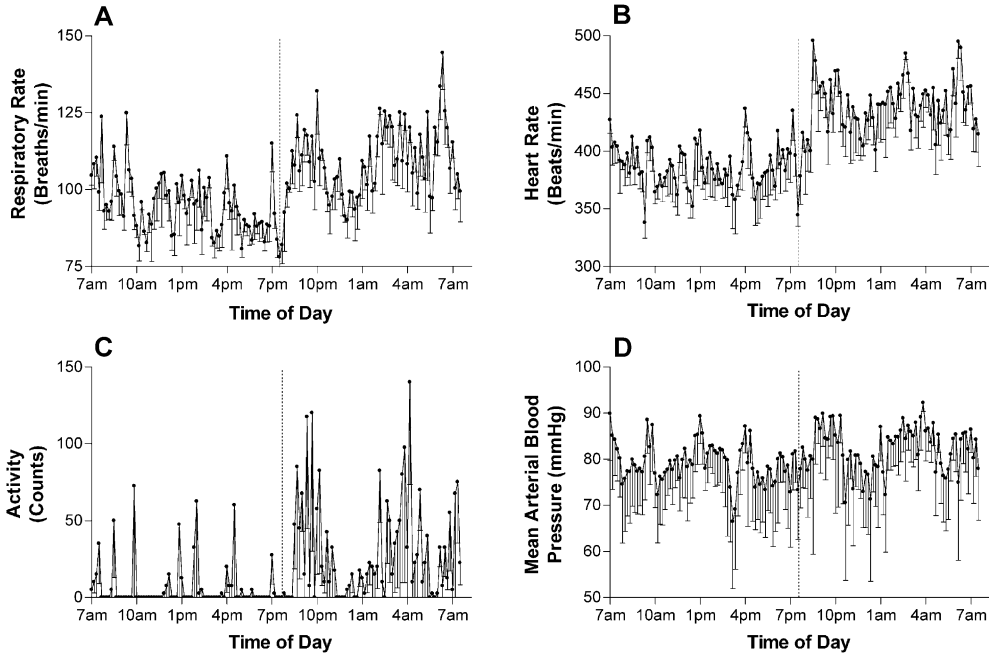

Fig. 1. Average (A) respiratory rate, (B) heart rate, (C) activity and (D) mean arterial blood pressure recordings taken on Day 4 of chronic methadone treatment (30 mg/kg/day). Mean ± S.E.M. ($n = 4$) were taken each min but data from every 10 min is shown. Dashed lines indicate lights on/off.

period ($P < 0.01$) (Fig. 2A). With methadone administration, the average day respiratory rates decreased, becoming significantly depressed from Day 2 onwards ($P < 0.01$). The induction of methadone treatment also produced respiratory depression throughout the night ($P < 0.01$). Significant differences between the day and night averages were also observed on Day 3 ($P < 0.05$) and Day 4 ($P < 0.01$) and were due to the average night respiratory rates increasing while the respiratory rates during the inactive day period remained depressed. Whilst the average respiratory rates during Night 3 and Night 4 were still significantly lower than baseline, they were significantly higher than on Night 2 ($P < 0.05$). This would indicate that partial tolerance was developing to the respiratory depressant effects of continuous methadone infusion during the night, but this was not evident in the day measurements.

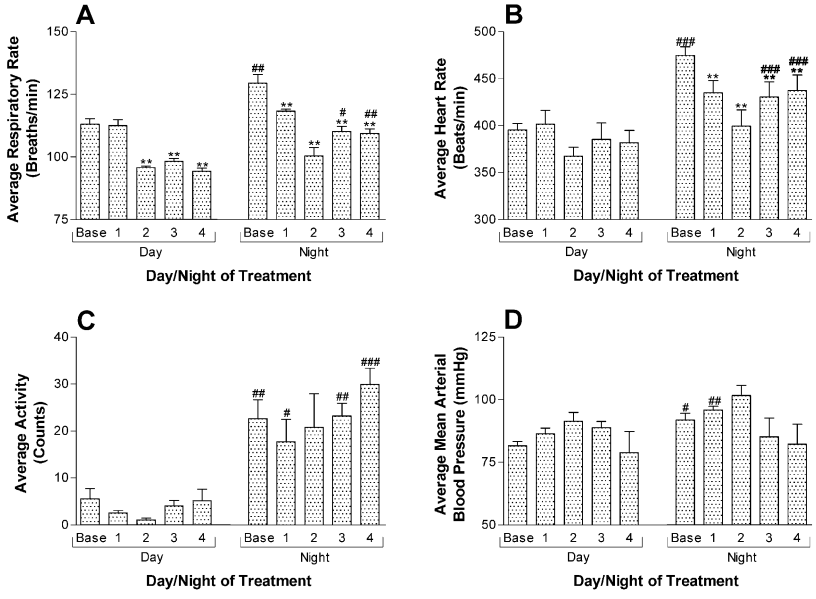

Fig. 2. Average (A) respiratory rate, (B) heart rate, (C) activity and (D) mean arterial blood pressure for day and night periods from baseline (Base) and Days 1 to 4 of chronic methadone treatment (30 mg/kg/day). $^{**}P < 0.01$ compared to day or night baseline, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ compared to same day average.

During the 24-h baseline recordings, heart rate was also significantly higher during the night than the day ($P<0.001$) (Fig. 2B). With chronic methadone infusion, average heart rate did not alter in the day period, but was significantly lowered during the night ($P<0.01$). As with respiratory rate, significant differences between the day and night averages on Day 3 and Day 4 were present ($P<0.001$). The average heart rates on Night 3 and Night 4 were still decreased compared to the baseline recordings, but significantly higher than during Night 2 ($P<0.05$, $P<0.01$, respectively). This would suggest that partial tolerance to the decreased heart rate occurred with this chronic methadone treatment.

As expected, locomotor activity was much higher throughout the night as rodents sleep during the day and are more energetic at night (Fig. 2C). The chronic methadone treatment did not produce any changes in the activity profile of these animals, with the activity remaining significantly higher during the night than the day and no changes being observed compared to baseline measurements.

There was very little change in the mean arterial blood pressure with the chronic methadone administration. Pretreatment blood pressure was higher during the night than in the day and this also occurred on Day 1, but was not significantly different after this time. There were also no changes in blood pressure from the baseline average in either the day or night.

In this study, we also examined the effect of antagonist administration on the changes produced with chronic methadone infusion. The average respiratory rate, heart rate, locomotor activity and mean arterial blood pressure 40 min after saline, naloxone hydrochloride (3 mg/kg i.p.) or naloxone methiodide (100 mg/kg i.p.) administration are shown in Fig. 3. Respiratory rate was not altered with saline administration and remained depressed compared to the average baseline measurements from the same time of day (Fig. 3A). Both naloxone hydrochloride and naloxone methiodide reversed this methadone-induced respiratory depression and also increased respiratory rate above the baseline pre-methadone average. Methadone administration decreased heart rate compared to baseline and saline did not alter this average, while naloxone hydrochloride and naloxone methiodide significantly increased this heart rate compared to both the saline average and the baseline average (Fig. 3B). Locomotor activity was low before methadone treatment and remained low after the administration of saline, whilst naloxone hydrochloride and naloxone methiodide increased this activity. This was only statistically significant with naloxone hydrochloride treatment (Fig. 3C). None of the treatments produced significant changes in mean arterial blood pressure, which remained the same as baseline throughout the methadone treatment (Fig. 3D).

4. Discussion

This to the first reported study that has used radiotelemetry to measure the effect of chronic methadone infusion on respiratory rate and the corresponding changes in heart rate, locomotor activity and mean arterial blood pressure in conscious unrestrained rats (Figs. 1 and 2). We have also examined the effect of naloxone hydrochloride and naloxone methiodide and confirmed that they are both effective in reversing opioid-induced respiratory depression in rats as

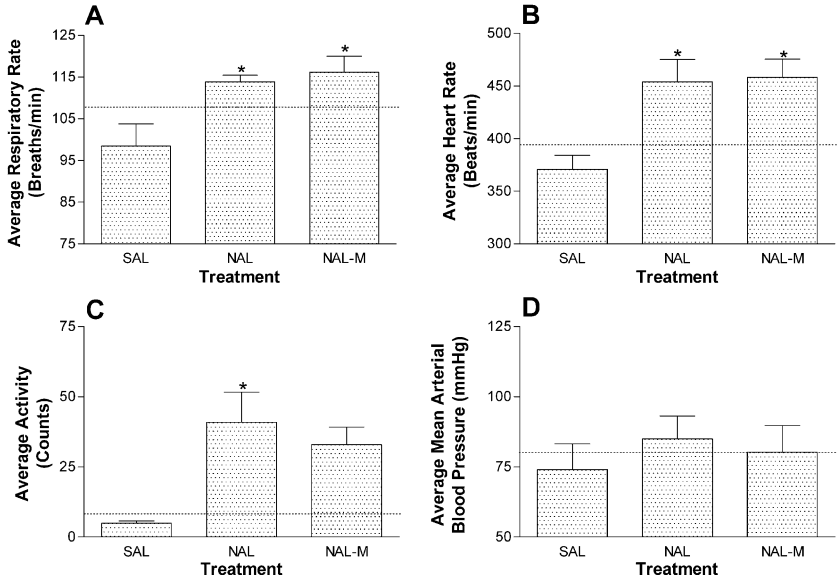

Fig. 3. Average (A) respiratory rate, (B) heart rate (C) activity and (D) mean arterial blood pressure over 40 min during baseline (no methadone) and after saline (SAL), 3 mg/kg i.p. naloxone hydrochloride (NAL) or 100 mg/kg i.p. naloxone methiodide (NAL-M) administration in animals treated chronically with methadone (30 mg/kg/day). *$P<0.05$ compared to saline. Mean ± S.E.M. ($n=4$) are shown. Dashed lines signify baseline, pretreatment recordings from the same period.

previously shown in mice (Fig. 3). The cardiovascular changes that occur with the administration of these opioid receptor antagonists have also been investigated (Fig. 3).

Chronic methadone administration does not appear to alter the normal circadian rhythm that has been previously observed in rats using radiotelemetry (Kramer et al., 1999). In particular, the circadian locomotor activity pattern did not alter, with activity remaining higher in the night than during the day and no changes compared to baseline activity being observed (Fig. 2C). Opioid administration in rats is known to produce hyperactivity in low doses and at high doses immobility followed by an increase in locomotor activity with continued treatment (Amalric and Koob, 1985). We did not observe either of these effects, which may be due to our constant infusion of methadone and the long period over which we monitored these rats.

Despite the lack of changes in activity observed in these animals, a significant decrease in respiratory rate was observed (Fig. 2A). The respiratory depression reported is, therefore, not primarily due to the animals being more sedentary upon opioid administration. Whilst respiration was depressed in both the active and inactive periods, normal circadian rhythms still existed. The increased respiration observed in the night may be due to increased activity over this period compared to the daytime period. However, on Night 2 respiratory rate was as low as during the day despite activity remaining high over the night period.

Our results also demonstrated partial tolerance to the respiratory effects of chronic methadone during the active nighttime period but not during the inactive daytime period. Existing literature does show a certain degree of tolerance to the respiratory depressant effects of methadone, but it does not appear to be complete (Dyer et al., 1999; Gritz et al., 1975; McCormick et al., 1984; Santiago et al., 1977; White and Zagon, 1979). No other studies have been conducted that allowed the observation of this type of effect on respiration, illustrating the usefulness of continuous radiotelemetry measurements. Our methadone treatment only continued for 4 days, but it would be of interest to extend this regime to further investigate this tolerance and determine if and when changes occur over the inactive period. These results indicate that the development of tolerance to these respiratory effects does not develop rapidly. This highlights the importance of carefully monitoring patients during induction onto opioids for either the treatment of chronic pain or maintenance therapies, particularly when long acting opioid receptor agonists, such as methadone, are administered.

We are unaware of any reports indicating that tolerance develops more rapidly during specific periods of the day. Evidence exists suggesting that opioid overdoses in humans occur more commonly in the night than during the day (Darke et al., 1996; McGregor et al., 2002; Wolff, 2002). Given that rats are at rest during the day and tolerance did not appear to develop during this treatment regime, it would follow that humans would be at most risk of opioid-related respiratory complications during their inactive night periods. Therefore, the timing of opioid overdoses may be due to circadian rhythms in respiration coupled with failure of tolerance to develop and may not only be a result of behavioural factors, such as a greater likelihood of overdose symptoms going unnoticed or greater use of opioids during the night than the day. This finding may have significant implications regarding our understanding of the mechanisms of opioid-induced respiratory depression and overdose.

The use of radiotelemetry has enabled us to determine the cardiovascular changes that occur with the chronic infusion of methadone. Previous research in our laboratory has shown that heart rate is decreased with constant infusions of 20 or 30 mg/kg/day morphine, but we have extended these findings to show that these decreases occur predominantly at night (Chan et al., 1999) (Fig. 2B). As with respiratory rate, tolerance to this depression occurred during the night periods, however this is difficult to confirm as we did not observe any changes in the daytime average heart rate. Unlike our previously reported morphine treatment, we did not observe any changes in blood pressure with methadone treatment (Chan et al., 1999) (Fig. 2D). This highlights the differences in effects between morphine and methadone, which may be due to the non-opioid effects of methadone.

This study was also designed to extend our previous research in mice and determine the effectiveness of naloxone hydrochloride and naloxone methiodide in reversing the effects observed in chronically methadone-treated rats. Respiratory rate was significantly increased with naloxone hydrochloride and naloxone methiodide treatment compared to saline treatment (Fig. 3A). The respiratory rates after antagonist treatments were also higher than the baseline average, but did not reach significance. This is in line with previous research that has shown that these opioid receptor antagonists can reverse the respiratory effects of opioids, but under normal breathing conditions do not significantly increase respiration above normal levels (Schlenker and Inamdar, 1995).

This study revealed that while heart rate was not altered by saline administration it was significantly increased with naloxone hydrochloride or naloxone methiodide treatment (Fig. 3B). These effects have been observed in previous studies using acutely opioid-treated rats but we have extended these findings to the chronically methadone-treated rat (Cruz and Rodriguez-Manzo, 2000; Czapla et al., 2000).

Our finding that naloxone methiodide, which is believed not to cross the blood–brain barrier, was as effective in reversing methadone-induced depression of respiratory rate and heart rate suggests that peripherally mediated mechanisms contribute to these opioid effects. Opioid receptors have been isolated in the heart and non-classical opioid receptors are thought to be present in the lungs which provides possible sites of action for naloxone methiodide (Barron, 2000; Cabot et al., 1994). Other cardiovascular effects, such as ischaemic preconditioning, are also thought

to involve peripherally mediated opioid effects so our results confirm that peripheral actions may play a significant role in mediating opioid-induced cardiovascular changes (Milanes et al., 2001; Schultz et al., 1997). The peripheral component of the cardiorespiratory actions of naloxone methiodide could also be further investigated using vagotomized animals, as previously shown with morphine and naloxone hydrochloride administration (Baraban et al., 1993).

Our results have illustrated that activity in these rats was significantly increased by treatment with naloxone hydrochloride and non-significantly increased by naloxone methiodide treatment (Fig. 3C). Whilst this result may be taken as these opioid receptor antagonists producing withdrawal, our previous studies have shown that naloxone methiodide does not produce withdrawal in opioid-treated mice (Lewanowitsch and Irvine, 2002). Activity is also not a direct measurement of withdrawal so we do not anticipate that these animals were undergoing significant withdrawal after naloxone methiodide administration, particularly as methadone was continually infused over these treatment periods (Schulteis et al., 1994).

This study has shown that radiotelemetry can be effectively used to measure changes in respiratory rate and cardiovascular parameters with chronic methadone administration. We have shown that partial tolerance appears to develop to decreases in respiratory rate and heart rate over 4 days, but this only occurs during the active night period. The lower respiratory rate and lack of tolerance development during the inactive period may explain why human opioid overdoses occur at a disproportionately high rate during the inactive (nighttime) part of the day. Naloxone methiodide was as effective as naloxone hydrochloride in reversing opioid-induced respiratory depression and these two antagonists significantly increased heart rate in chronically methadone-treated rats. This suggests a peripheral component to the cardiorespiratory effects of opioids that may be targeted in the future treatment or prevention of opioid overdoses. Radiotelemetry is, therefore, an attractive alternative to other respiratory and cardiovascular measurement techniques available to determine the pharmacological alterations that occur with chronic drug treatment regimes.

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