A telemetric examination of cardiovascular function during the development of, and recovery from, opiate dependence in rats

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Abstract

Rats were subject to daily injections of morphine or saline and were then allowed to spontaneously withdraw from morphine for 4 days. Mean arterial blood pressure (MAP) and heart rate (HR) were recorded continuously, via radiotelemetry, during the development of, and recovery from, opiate dependence. Injections of morphine produced pronounced and prolonged increases in MAP and HR which increased as morphine dose increased. There were also significant increases in MAP during the 19–23 h period after each morphine injection indicating the presence of withdrawal. Spontaneous withdrawal from morphine was associated with a pronounced (20% increase from baseline) and prolonged (72 h) increase in MAP. MAP returned to baseline levels 72–96 h after last morphine exposure. These results show that intermittent injections of morphine, and spontaneous withdrawal from these injections, are associated with profound alterations in cardiovascular function and confirm the usefulness of radiotelemetry for studying opiate dependence.

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1. Introduction

Prolonged administrations of opiates produce the development of tolerance and withdrawal. The withdrawal syndrome from opiates in humans includes cognitive (e.g., drug craving), affective (e.g., anxiety), and behavioural (e.g., twitches) signs [16]. Changes in autonomic function are also key indicators of opiate withdrawal. These can include cardiovascular alterations (e.g., heart rate and blood pressure), changes in respiration, and changes in thermoregulation [6].

Despite the important place of autonomic changes in the opiate withdrawal syndrome, there have been few investigations into autonomic function during dependence and withdrawal from opiates using animal models [1,2,4,17]. Chan et al. [4] implanted rats with radiotelemetry probes to continuously monitor blood pressure and heart rate in the freely moving animal. They also implanted rats with minipumps to continuously deliver morphine. They showed that delivery of morphine produced small increases in blood pressure which displayed tolerance across prolonged exposures. Precipitation of withdrawal via removal of the minipumps also resulted in a small but significant increase in blood pressure as well as heart rate. Similar increases in blood pressure have been reported in rats following antagonist-precipitated withdrawal [1,2,17] and in humans during spontaneous [10,11,14] and antagonist-precipitated [6] withdrawal. The relative lack of research into the autonomic correlates of opiate dependence and withdrawal in animals is surprising because investigations into the patterns of neural activation associated with opiate withdrawal consistently identify pronounced activation of central autonomic circuits. For example, it is well documented that withdrawal from opiates induces immediate early gene expression in structures such as the nucleus of the solitary tract, rostral and caudal ventrolateral medulla, A5 catecholaminergic group, parabrachial nuclei, locus coeruleus, paraventricular nucleus of the hypothalamus, amygdala and thoracic spinal cord [8,9,13,15,18,19,22,23], all of which play critical roles in regulating autonomic function.

Although past studies have identified robust changes in autonomic function during opiate withdrawal, they have also left unanswered a number of important questions. First, the time
course of the development of opiate-induced changes in autonomic function is unclear because none of these studies have recorded continuously across a prolonged period of opiate injections. Second, the time course of recovery from opiate-induced alterations is unclear because only one study has recorded autonomic changes continuously across a prolonged period of withdrawal from opiates [4]. Typically intervals of 24 h or less have been studied [1,2,17]. Thirdly, the effects of intermittent exposures to opiates, and withdrawal from these exposures, on autonomic function are unclear because prior studies have typically employed constant infusions of opiates [1,4]. This is of considerable significance because administration regimes employing constant infusions of opiates bear little resemblance to patterns of human opiate use. The typical pattern of human opiate use is frequent, often daily, episodes of intoxication and patterns of human opiate use. The typical pattern of human opiate use is frequent, often daily, episodes of intoxication and withdrawal rather than continuous opiate exposure followed by a single episode of opiate withdrawal [7]. Finally, the effects of spontaneous withdrawal from opiates on autonomic function are unclear because these experiments have typically studied antagonist-precipitated withdrawal from opiates [1,2,17]. Indeed, only two prior studies have characterised autonomic parameters during spontaneous withdrawal from opiates in rats [4,17] but the interpretation of these results is limited by the fact they involved continuous exposures to morphine to induce dependence.

The aim of the experiment reported here was to study autonomic function during intermittent exposures to opiates and during spontaneous withdrawal from these exposures. Rats, implanted with telemetric probes in the descending aorta permitting continuous recording of mean arterial blood pressure (MAP) and heart rate (HR), were injected daily for 13 days with either morphine or saline. The doses of morphine increased from 2.5 to 20 mg/kg. The rats then remained undisturbed in their home cages for a further four days to permit spontaneous opiate withdrawal.

2. Method

2.1. Subjects

Subjects were eight male Wistar rats weighing between 300 and 325 g at the start of the experiment. They were obtained from a commercial supplier (Gore Hill Research Laboratories, Sydney, Australia). Prior to the start of the experiment, rats were housed in groups of 8 in plastic cages (67×40×22 cm [L×W×H]) maintained under natural lighting. They were handled for 5 days prior to the start of the experiment. At the commencement of the experiment rats were singly housed in plastic cages (40×26×16 cm [L×W×H]) in a quiet room maintained under natural light. Food and water were freely available for the duration of the experiment. The procedures used were approved by the University of New South Wales Animal Care and Ethics Committee.

2.2. Apparatus

2.2.1. Telemetry

Individual rat cages were placed on top of receivers for concurrent measurement of heart rate (HR) and mean arterial blood pressure (MAP). HR and MAP were extracted automatically from the pulsatile blood pressure signal using the DATAQUEST software (Data Sciences International). HR and MAP were sampled every 120 s from 3-s time windows.

2.2.2. Drugs

Rats were injected daily subcutaneously (s.c.) in the dorsal neck region daily with morphine HCL (a generous gift from Glaxo-Wellcome) (n=4) or saline (n=4) or sterile non-pyrogenic saline (0.9% w/v). We employed an ascending dose regime so that rats were injected with 2.5 mg/kg for three days, 5.0 mg/kg for three days, 10.0 mg/kg for three days, and 20.0 mg/kg for four days. This injection regime was chosen because previous work has shown that it produces tolerance and withdrawal [24]. All injections volumes were 1 ml/kg.

2.3. Procedure

2.3.1. Surgery

Rats were injected i.p. with 1.3 ml/kg of the anaesthetic ketamine (Ketapex; Apex Laboratories, Sydney, Australia) at a concentration of 100 mg/ml, and with 0.3 ml/kg of the muscle relaxant xylazine (Rompun; Bayer, Sydney, Australia) at a concentration of 20 mg/ml. After the onset of stable anaesthesia rats were implanted with radiotelemetry devices (TA11PA-C40, Data Sciences, St. Paul, MN) as described by Carrive [3]. Briefly, a midline incision was made in the abdomen and the descending aorta was exposed at the level of the iliac bifurcation. The artery was punctured at this level and the fluid-filled sensor catheter was inserted and fixed in place with tissue adhesive (3M, Animal Care Products, St. Paul, MN). The body of the probe was immobilized by suturing to the abdominal wall and the skin wound was closed with suture clips. The rats were injected s.c. with 5 mg/kg of Carprofen to provide pain relief and 0.33 ml procaine penicillin to prevent infection and moved to individual plastic home boxes located in a sound proofed room in which they were housed for the duration of the experiment. The rats were allowed 7 days recovery prior to the start of the experiment.

2.3.2. Baseline recordings

The animals remained undisturbed in their home cages for three days. During this time MAP and HR were sampled every 2 min. These recordings served as baseline recordings.

2.3.3. Dependence induction

Each day at 10:40 AM the experimenter entered the room, briefly removed the rats from their home cages, injected them with saline or morphine according to group allocations, and then left the room. The rats remained otherwise undisturbed in their home cages.

2.3.4. Spontaneous withdrawal

At the end of the 13 days of morphine and saline injections the rats remained undisturbed in their home cages for 4 more days.
2.4. Data analysis

The data were prepared as follows. For each animal an average was calculated for MAP and HR for each hour of the experiment (3 days baseline, 13 days morphine, 4 days recovery). For each animal an average for each hourly period (e.g., 10:40–11:40; 11:40–12:40; 12:40–13:40, etc) for the three days of baseline recording was then calculated. This average served as a baseline for subsequent transformations. Thus, baseline data was the hourly average MAP and HR across the three days. MAP and HR for each animal for each hour of the 13 days dependence induction and the 4 day recovery period was calculated as a percentage change from the corresponding time of day during baseline. For example, the MAP and HR response for the hour following injection of morphine or saline on Day 1 (10:40–11:40) was expressed as percentage change from the 10:40–11:40 baseline. Data were converted to a measure of percentage change from baseline because: 1) it reveals opiate and opiate withdrawal effects independently of diurnal variations in HR and MAP; 2) it has the potential to reveal more clearly changes in HR and MAP across time (e.g., development of hypertension); 3) it enables direct examination of any dose-dependent effects of morphine or morphine withdrawal in HR and MAP; and 4) each animal serves as its own control. These data were then analysed via repeated measures ANOVA.

3. Results

There were no differences between groups in either HR (mean HR group Saline=370 bpm; SEM=6; group Morphine=370 bpm; SEM=5) (p>0.05) or MAP (MAP group Saline=100 mm Hg; SEM=3; group Morphine 107 mm Hg; SEM=3) (p>0.05) averaged across the three days of baseline recordings.

Fig. 1 shows the mean and standard error of the mean (SEM) HR (top panel) and MAP (bottom panel), expressed as percentage change from baseline, across the 13 days of saline or morphine injections. Each data point represents the average of a 1 h recording period. There are a number of interesting features of the data. First, it is clear that injections of morphine produced pronounced increases in HR and MAP. The size of these increases appeared to be related to duration and/or dose of morphine: as duration and dose increased so too did HR and MAP. Second, it is clear that these increases in HR and MAP were also prolonged, persisting for at least 5 h following injection (each data point represents a 1 h recording period). Third, it is clear that both HR and MAP recovered within 6–8 h of injection of morphine.

In order to analyse the influence of morphine and saline on autonomic function we studied the peak change in HR and MAP, expressed as percentage change from baseline, during the 5 h immediately following injection of saline or morphine.

![Fig. 1. Mean and SEM changes in heart rate (top panel) and arterial blood pressure (bottom panel) recorded continuously across 13 days of injections of morphine (n=4) or saline (n=4). Data expressed as percent change from the corresponding time of day during baseline recordings. All injections were given at 10:40. Each data point corresponds to the average of a 1 h measurement period.](image-url)
These data are shown in Fig. 2. Inspection of the figure indicates pronounced increases in HR (20–40% above baseline) and MAP (10–30% above baseline) following morphine injection. For HR, a repeated measures ANOVA revealed a significant main effect of Group ($F(1, 6)=33.3; p<0.05$), indicating that injections of morphine significantly increased peak HR. There was no overall significant main effect of Time ($F(12, 72)=1.5; p>0.05$). Importantly, there was a Group×Time interaction ($F(12, 72)=6.2; p<0.05$) indicating an increase in peak HR across days among morphine but not saline injected rats, so that the morphine-treated rats showed a significantly greater increase in peak HR across days of injections. Similar results were found with peak MAP. A repeated measures ANOVA revealed a significant effect of Group ($F(1, 6)=23.2; p<0.05$), indicating that injections of morphine significantly increased peak MAP. There was an overall significant effect of Time ($F(12, 72)=1.9; p<0.05$). There was a Group×Time interaction ($F(12, 72)=3.9; p<0.05$) so that the morphine-treated rats showed a significantly greater increase in peak MAP across days of injections.

To assess the development of withdrawal during the 13 days of injections we calculated the average change in HR and MAP during the 4-h period 19–23 h after last injection. This data is shown in Fig. 3. For HR, a repeated measures ANOVA revealed a significant effect of group ($F(1, 6)=6.2; p<0.05$). Inspection of the figure indicates higher HR in morphine-treated rats compared to saline-treated during this period but this increase was only slight and was not substantially above baseline. There was no overall significant effect of Time ($F(12, 72)=1.8; p>0.05$) and there was no Group×Time interaction ($F(12, 72)=1.0; p>0.05$). The results from MAP were clearer and more pronounced. A repeated measures ANOVA revealed a significant effect of group ($F(1, 6)=53.0; p<0.05$), indicating significant increases in MAP during the 19–23 h period after each injection. There was also an overall significant effect of Time ($F(12, 72)=2.5; p<0.05$). Finally, there was a Group×Day interaction ($F(12, 72)=3.3; p<0.05$) so that the change in mean MAP across days of injections was significantly greater among morphine- than saline-treated rats.

Finally, Fig. 4 shows the mean and SEM HR (top panel) and arterial blood pressure (bottom panel), expressed as percentage change from baseline, during the 4 days of spontaneous withdrawal from morphine. Inspection of the figure shows that spontaneous withdrawal was associated with a pronounced and prolonged increase in MAP and little change in HR. This increase in MAP persisted for at least 72 h and MAP had returned to control levels within 96 h. The statistical analysis confirmed these observations. For HR, the repeated measures ANOVA revealed no significant effect of group ($F(1, 6)=1.1; p>0.05$). There was an overall significant effect of Time ($F(9, 564)=1.6; p<0.05$) and there was a Group×Time interaction ($F(9, 564)=1.7; p<0.05$). From inspection of the figure, this suggests that there was a small decrease in HR across the withdrawal period among the morphine-treated rats compared to the saline-treated rats. Again, the changes in MAP were more pronounced. For MAP
the repeated measures ANOVA revealed a significant effect of Group ($F(1, 6)=49.5; p<0.05$), a significant effect of Time ($F(94, 564)=3.9; p<0.05$), and a significant Group×Time interaction ($F(94, 564)=3.7; p<0.05$). These results indicate that spontaneous withdrawal from morphine was associated with a significantly elevated MAP which decreased across days.

4. Discussion

This experiment studied cardiovascular function during the development of, and recovery from, opiate dependence. The results can be summarised succinctly. Injections of morphine produced pronounced (25–40% above baseline) and prolonged increases in MAP and HR. These alterations were more pronounced as the duration and dose of opiate exposure increased. MAP also increased significantly during the 19–23 h period following each injection. These increases in MAP were also more pronounced as the duration and dose of opiate exposure increased. There were smaller, but significant, group differences in HR during the 19–23 h period following each injection. Finally, spontaneous withdrawal from morphine was also associated with a pronounced (25%) and prolonged (72 h) increase in MAP and no overall group differences in HR.

Injections of morphine produced pronounced increases in MAP and HR. The size of this increase was related to the dose and/or duration of opiate exposure. Although the present experiment documented robust evidence for increases in MAP and HR following subcutaneous administrations across a wide dose range, the effects of morphine on cardiovascular function in rats are complex. Prior research has shown that continuous subcutaneous administrations of morphine, via implantation of minipumps, produced significant increases in systolic and diastolic blood pressure on the first day of morphine treatment but dose-dependent decreases in heart rate, systolic and diastolic blood pressure thereafter [4]. By contrast, acute intravenous morphine produces a transient increase in MAP and long-lasting bradycardia [5] whereas acute intrathecal morphine produces a brief and small reduction in MAP [12]. These findings suggest that the direction, magnitude, and duration of morphine’s effects on cardiovascular function depend critically on the route and method of administration.

In contrast to the effects of morphine, the effects of withdrawal from morphine on cardiovascular function appear independent of the route and method of administration used to induce dependence. The present results show for the first time that spontaneous withdrawal from intermittent injections of morphine is associated with pronounced and prolonged increases in MAP. These results are consistent with previous research using either spontaneous or antagonist-precipitated withdrawal from continuous exposures to subcutaneous or intravenous morphine [1,2,4,17]. Although it is difficult to make comparisons across different morphine administration regimes, it is worth noting that the magnitude of the increase in MAP during spontaneous withdrawal here was substantially larger than that previously reported. The spontaneous withdrawal from intermittent injections of morphine here was associated with an approximately 20% increase in MAP and persisted for approximately 72 h. Previously spontaneous withdrawal from continuous exposures to morphine was associated with a much smaller increase (approximately 5%) [4] and only antagonist-precipitated withdrawal produced increases in MAP of 20% or greater [1]. The present results show therefore that spontaneous withdrawal from daily intermittent injections of morphine is associated with profound and prolonged alteration in cardiovascular function.

Perhaps the most interesting finding from this experiment was the emergence of cardiovascular changes in the 19–23 h period following each morphine injection. MAP increased following injection of morphine and then returned to levels of controls before significantly increasing again 19–23 h after last injection. There was also an overall difference in HR between groups during this period, but this difference was small and the increase in morphine-treated rats was not substantially above baseline. The increases in MAP during the 19–23 h post-injection period were more pronounced as the duration and/or dose of opiate exposure increased. Because these changes in MAP occurred at a prolonged interval since last injection, and because they increased as the duration and/or dose of opiate exposure increased, one interpretation is that they reflect spontaneous withdrawal from the injection given 19–23 h previously. However, another possibility is that these changes in MAP represent anticipation of the impending morphine injection, that is, they reflect the operation of conditioning processes. There is compelling evidence for the operation of conditioning processes during repeated opiate administrations [21], including conditioning of autonomic responses [20]. In the
present experiment all injections were given at the same time of day (10:40). According to a conditioning analysis, the alterations in MAP observed in the hours prior to morphine injection are conditioned responses emerging from learning about stimuli, presumably temporal cues including variations in lighting, which signal the arrival of morphine in the body. Indeed, these two possibilities are not mutually exclusive. The MAP responses observed during this period could reflect summation of both non-associative (spontaneous withdrawal) and associative influences. This result is interesting for at least two reasons. It is the first time that the development of such MAP responses has been recorded on a continuous basis across repeated exposures to an opiate. Second, the strength of these MAP responses increases as the duration and/or dose of opiate exposure increase. The relationship between these autonomic changes and propensity to engage in drug-seeking and drug-taking could be worth examining.

Finally it is worth noting that this experiment did not attempt to separate the influence of duration of opiate exposure and dose of opiate exposure on the development of cardiovascular changes. In other words, the alterations in autonomic function documented here during the thirteen days of opiate treatment and the four days of recovery could have been due to the increasing doses of opiate (2.5–20 mg/kg) or increasing duration of opiate exposures (1–13 days) or both. Identifying the separable or interacting influences of these two variables could be a focus for future research. Regardless, this experiment shows for the first time that pronounced changes in autonomic function occur during intermittent exposures to morphine and spontaneous withdrawal from these exposures and they highlight the usefulness of the telemetric approach for studying these changes.

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References