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BREATHING AND TEMPERATURE CONTROL DISRUPTED BY MORPHINE AND STABILIZED BY CLONIDINE IN NEONATAL RATS

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Abstract

Background—Sedative-analgesics are often given to newborn infants and are known to affect many components of the autonomic nervous system. While morphine is most frequently used, α -2 adrenergic receptor agonists are being increasingly used in this population. Alpha-2 adrenergic receptors agonists also have anti-shivering properties which may make it a desirable drug to give to infants undergoing therapeutic hypothermia. The aim of this study was to systematically compare two different classes of sedative-analgesics, morphine, a μ -opioid receptor agonist, and clonidine an α -2 adrenergic receptor agonist on breathing, metabolism and core body temperature (CBT) in neonatal rodents.

Methods—Breathing parameters, oxygen consumption (VO_2) and carbon dioxide production (VCO_2), were measured prior to, 10 and 90 minutes after intraperitoneal (IP) administration of morphine (2, 10 or 20mg/kg), clonidine (40, 200 or 400 $\mu\text{g/kg}$), or saline in Sprague-Dawley rat pups at postnatal day 7 (p7) while continuously monitoring CBT.

Results—Morphine reduced the respiratory rate, VO_2 and VCO_2 greater than clonidine at all dosages used ($p < 0.05$, morphine vs. clonidine, for all metabolic and respiratory parameters). Furthermore, morphine induced prolonged respiratory pauses, which were not observed in animals treated with clonidine or saline. Morphine caused hypothermia which was dose dependent, while clonidine stabilized CBT in comparison to saline treated animals ($p < 0.0001$).

Conclusion—In the newborn rat, morphine causes profound respiratory depression and hypothermia while clonidine causes minimal respiratory depression and stabilizes CBT. All

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together, we suggest that clonidine promotes autonomic stability and may be a desirable agent to use in infants being treated with therapeutic hypothermia.

1. INTRODUCTION

Analgesia and sedation are an integral part of critical care medicine. Sedative-analgesics reduce the stress response, provide anxiolysis, facilitate respiratory support and optimize pain control. Opiates, specifically morphine, are the mainstay of pain control and sedation protocols in critically ill patients including newborns, despite having the undesirable side effect of respiratory depression (Niesters et al 2013). Alpha₂-adrenergic receptor agonists such as clonidine and dexmedetomidine, also provide analgesia, anxiolysis, and sedation, with the advantage of causing much less respiratory depression than opiates. Thus, the use of these drugs in critically ill newborns has increased significantly in the past years (Pichot et al 2012). Opiates often cause apnea thus prolonging need for mechanical ventilation (Hall et al 2005). In addition to its effects on breathing, morphine also decreases oxygen consumption (VO₂) in adults (Hurle et al 1985), and causes either hypothermia, hyperthermia or both (Geller et al 1983). In adults, clonidine, also depresses ventilation (McCrimmon & Lalley 1982), lowers body temperature (Ma et al 2004) and metabolic rate via its effects on the sympathetic nervous system (Gazzola et al 1995).

Despite the fact that both morphine and clonidine modify breathing, affect core body temperature (CBT) and metabolism in adults, the effects of these agents on autonomic parameters in newborns have not been systematically studied. The aim of this study was to determine the effect of morphine and clonidine on breathing, metabolism and CBT in a newborn rat at postnatal day 7 (p7) - a species and age commonly used to study newborn physiology. Thus, understanding the respiratory/metabolic side effects of these commonly used drugs in this pre-clinical model is important. Using the p7 rat pup, we tested the hypothesis that morphine and clonidine differentially affect respiratory parameters while lowering CBT and metabolic rate in awake, freely moving neonatal rodents.

2. MATERIALS AND METHODS

2.1 Animals

All animal studies were approved by the Animal Care and Use Committee of Johns Hopkins University School of Medicine and were performed in accordance with the NIH 'Guide for the care and use of laboratory animals' of the U.S. Department of Health and Human Services 85-23, 2011. Time dated Sprague-Dawley rat pups at p7 of both sexes were used. All animals were housed in the animal care facility. The rat pups were reared by the mother until the day of the experiment.

2.2 Experimental Design

Experiments were performed in un-anaesthetized and unrestrained rat pups at p7 (n=20/treatment group). The temperature of the chambers were servo controlled to maintain chamber temperature between 31–33°C and three different dosages of morphine (2, 10 and 20 mg/kg) and clonidine (40, 200 and 400µg/kg) were used. While these doses were selected based on previous publications that demonstrated neuroprotection and/or temperature

modulation (Ammon-Treiber et al 2004, Hoffman et al 1991, Yuan et al 2001), the range for analgesic-sedative dose in rats is 1–24mg/kg for morphine (Kawaraguchi et al 2008, McLaughlin et al 1990) and 50–1000 µg/kg for clonidine (Sakamoto et al 2013, Skingle et al 1982).

Experimental setup: CBT was measured using rodent rectal probe thermometers (model IT 18, Physitemp Instruments INC., Clifton, NJ, USA) attached to a T-type pod (model ML312, AD Instruments, Colorado Springs, CO, USA). The analog signal was transmitted to a Power Lab 6 data acquisition system (AD Instruments) and the output was analyzed with Chart Software (AD Instruments). The thermometer probe was lubricated and inserted rectally in gently restrained, un-anaesthetized rat pups. The rat pups were then placed in a Plexiglas thermo-neutral chamber and allowed to acclimate to the probe and the chamber. Temperature in the chamber was maintained between 31–33°C using servo-controlled heating elements that were constructed in house. After a period of stabilization (15–20 min) at which time the rat pups were quietly resting and the CBT had stabilized, the animal was removed from the chamber and given drug or saline and immediately placed back into the chamber. CBT was measured at baseline prior to the drug/saline administration and continuously for 90 min after intraperitoneal (IP) administration of drug/saline. Breathing and metabolic parameters were obtained using whole body plethysmography. The plethysmography setup was constructed in house and consisted of 4 Plexiglas chambers. This allowed for simultaneous measurement on four animals. A pressure transducer (model 8510B-2, Endevo, San Juan Capistrano, CA) attached to the plethysmograph generated an analog signal, that was transmitted to a Power Lab 6 data acquisition system (AD Instruments) and the output was analyzed with Chart Software (AD Instruments). Ventilatory parameters: respiratory rate [RR], tidal volume [V_T], inspiratory time [T_i] and inter-breath interval (including expiratory time) [IBI] were measured prior to drug/saline administration and at 10 and 90 min after treatment. Oxygen consumption (VO_2) and carbon dioxide (VCO_2) production were measured using an indirect open circuit calorimetric system (Oxymax Deluxe; Columbus Instruments, Columbus, OH, USA) (Tankersley et al 2007).

2.3 Drug Administration

The following drugs were used: morphine sulfate (MOR₂-2 mg/kg, MOR₁₀-10 mg/kg and MOR₂₀-20 mg/kg) from Baxter Health Care Corp. (Deerfield, IL, USA) and Clonidine hydrochloride (CLON₄₀-40 µg/kg, CLON₂₀₀-200 µg/kg and CLON₄₀₀-400 µg/kg) from Sigma Chemical Co. (St. Louis, USA). The animal was weighed, the abdomen was cleansed with alcohol, and the drug/saline was injected IP in a standard volume of 200µl. All drugs were diluted with sterile saline.

2.4 Statistical Analysis

Ventilatory and metabolic parameters (CBT, RR, T_i and IBI) were measured and calculated using the IGOR Software and plotted as scatter, line or bar graphs. Data were analyzed using 2-way analysis of variance (2-ANOVA) and paired t-tests (GraphPad PRISM, version 4 statistical software, GraphPad Inc., La Jolla, CA). Significance was defined by a p value <0.05. Data are presented as means ± SE.

3. RESULTS

3.1 Effect of morphine and clonidine on breathing parameters

RR, T_i and IBI parameters were analyzed prior to and 10 and 90 min after drug administration. We analyzed 10 consecutive breaths from the most stable period during a 2 min recording. RR and the pattern of breathing were affected by treatment with morphine and clonidine when compared to animals treated with saline at similar time points (representative tracings from 3 animals shown, Figure 1). However, morphine markedly affected the pattern of breathing with intermittent periods of prolonged pauses. To assess if this was a dose dependent effect, we tested, at 10 and 90 min after drug administration, the RR for different doses of morphine (2–20 mg/kg) and clonidine (40–400 μ g/kg) (Figure 2). In comparison to saline treated animals, both morphine and clonidine treated animals, at all doses studied, had significantly lower RR at 10 and 90 min after drug administration (Figures 2). Compared to baseline, MOR_{10, 20} reduced RR by 85% ($86 \pm 1.9\%$) and 90% ($89 \pm 1.1\%$) at 10 min, respectively, with no additional change by 90 min (Figures 2a, b). MOR₂ reduced RR by 45% ($45 \pm 12\%$) from baseline, which was only evident at 90 min (Figure 2b). CLON_{200, 400} decreased RR by 30 ($32 \pm 3\%$) and 50% ($50 \pm 1.4\%$), respectively, at 90 min, in comparison to baseline. CLON₄₀ transiently decreased RR at 10 min (Figures 1, 2) but RR returned to baseline by 90 mins. At baseline, all animals had a RR of approximately 182 ± 4.9 breaths/min. Ten minutes after drug administration, RR in MOR₁₀ pups was 27 ± 3.9 breaths/min and in MOR₂ pups 106 ± 17.2 breaths/min, such that 85% and 40% of the time, MOR₁₀ and MOR₂ animals, respectively were having respiratory pauses (prolonged IBI) (Figure 5). At 90 mins, in MOR₁₀ pups, RR remained low at 28 ± 1.2 breaths/min and MOR₂ pups had RR of 77 ± 17 breaths/min.

The reduction in RR in the drug treated animals was primarily caused by an increase in IBI vs. T_i . Both morphine and clonidine treated animals had significantly prolonged IBI in comparison to saline treated animals at similar time points. MOR₁₀ caused a marked increase in IBI after 10 and 90 min of drug administration leading to prolonged respiratory pauses (Figure 3). Compared to baseline, in MOR₂ treated pups IBI was unaffected at 10 min and prolonged by 90 min but in MOR₁₀ treated rat pups, IBI was increased at both time points (Figure 3b). CLON₄₀ had a minimal and transient effect on IBI at 10 min (Figure 3b). CLON₂₀₀ increased IBI significantly at 10 min, which remained prolonged even at 90 min compared to baseline. MOR₁₀ caused greater variability in IBI than the MOR₂ or CLON_{40, 200}; with a median of 2.5s and range of 0.3–11s (Figures 4c, 4d). Treatment affected T_i much less than IBI with T_i being slightly but significantly increased in MOR_{2, 10} and in CLON₂₀₀ animals (Figures 3a, 4a, 4b). Of note, data for MOR₂₀ and CLON₄₀₀ is not shown since the change in RR at the highest doses for both the drugs was no different from their respective intermediate doses (MOR₁₀ and CLON₂₀₀).

In morphine treated pups, RR was profoundly reduced, with infrequent inspiratory breaths that initially appeared to be gasping because visually the animal also had a myoclonic jerk associated with each breath. However the shape of the inspiratory breath had a slower rate of rise in contrast to the rapid rate of rise which characterizes gasps. We compared the breathing tracing from the morphine exposed animals to that of tracings from non-sedated,

un-anaesthetized rat pups also at p7 during exposure to 95% N₂ and 5% CO₂ (Figure 5). Gasping breaths have a rapid inspiratory rise with a retarded expiratory phase preceded and followed by a cessation of breathing movements. Breathing pattern of morphine treated pups continued to resemble eupneic breaths though interspersed with long respiratory pauses. However, in a subset of the morphine exposed animals placed back with the dam, 18 hrs later the breathing pattern had returned to normal. Thus, in newborn rat pups, both drugs in the dosages used in this study decreased RR, but only morphine exposure was associated with multiple episodes of prolonged respiratory pauses.

3.2 Effect of treatment on CBT, VO₂ and VCO₂

CBT fluctuated during the first 15 min after treatment, began to stabilize by 30 min and showed no further changes after 60 min in all animals. CBT did not increase in morphine and clonidine treated rat pups, ($p < 0.0001$, ANOVA). Rat pups treated with MOR₁₀ or CLON_{200, 400} maintained their CBT throughout the experiment while those treated with MOR₂₀, dropped their CBT by 2.8°C from baseline by 60 min ($p < 0.0001$ baseline vs. 60 min), (Figure 6a). The effect of MOR and CLON on CBT also differed; MOR₂₀ reduced CBT when compared to CLON_{40, 200, 400} ($p < 0.05$, MOR₂₀ vs. CLON_{40, 200, 400}). MOR₂ and CLON₄₀ did not change CBT from baseline (Figure 6b). Thus, clonidine at all dosages and morphine at moderate dosages maintained CBT, however morphine at the highest dose induced hypothermia, decreasing CBT by ~ 3 degrees by 90 min after administration in newborn rats (Figure 6a, 6b). We speculate that the increase in CBT observed in the saline treated animals might be stress induced since the pups were removed from the mother and placed in a novel environment (Dallmann et al 2006, Ito et al 2006, Yee et al 2011). Although not systematically measured, both clonidine and saline animals had similar levels of activity, thus, it is less likely that exploring a novel environment is the etiology for the differences in temperature elevation.

VO₂ and VCO₂ after morphine and clonidine treatment was significantly lower ($p < 0.0001$ for both VO₂ and VCO₂ treatment vs. saline), (Figures 7a, 7b). MOR_{2, 10, 20} treatment lowered VO₂ at 90 min by 60, 80 and 90% and VCO₂ by 42, 65, and 80% respectively in comparison to saline treatment (Figures 7c, 7d). Only the animals treated with the highest dose of clonidine had VO₂ and VCO₂ measurements that differed from saline treated controls at 90 min (VO₂ was reduced by 50% and VCO₂ by 45% compared to saline controls (Figures 7c, 7d). The effect of morphine and clonidine on VO₂ and VCO₂ also differed; MOR₂₀ significantly reduced VO₂ and VCO₂ compared to CLON_{40, 200} but not when compared to CLON₄₀₀ (Figures 7c, 7d). The effect of morphine and clonidine on VO₂ and VCO₂ persisted for 90 min. While both drugs reduced VO₂ and VCO₂, morphine had a greater effect on VO₂ and VCO₂ in newborn rat pups.

4. DISCUSSION

In the present study, we show for the first time that clonidine had minimal effect on breathing and metabolism while maintaining the CBT in a p7 newborn rat. In contrast, morphine caused a reduction in metabolism and had a profound effect on breathing leading to significant and prolonged IBI with prolonged respiratory pauses; and at the higher dose induced hypothermia. Morphine and clonidine are analgesic-sedative-anxiolytics that are

commonly used to treat critically ill newborn infants. The importance of proper sedation in the neonatal ICU setting (Peters et al 2005) and maintenance of sedation during therapeutic hypothermia for the treatment of HIE to achieve neuroprotection (Gressens et al 2008) have been demonstrated. Sedatives affect multiple physiologic variables that could exacerbate or inhibit neurologic injury. Other than their relatively well known effects on respiratory rate, little is known about the effects of these commonly used drugs on temperature, metabolism and respiratory parameters in the neonatal model. Thus, this study was designed to determine the effect of two classes of sedative-analgesics on these physiological parameters in an animal model commonly used to study neonatal physiology and neonatal brain injury (Northington 2006, Rice et al 1981).

Opioid receptors (μ , δ , κ) are found in the nTS and Pre-Bötzinger complex (PreBötzc) areas that control inspiratory rhythmogenesis. Even at low doses, opioids affect the rhythm generation center in the Pre-Bötzc in the medulla (Gray et al 1999) causing respiration to slow and become irregular (Leino et al 1999). μ -opioid receptor agonists depress tidal volume and gas exchange, slow the rate of breathing, and ultimately cause respiratory arrest. The respiratory depression induced by opioids is greater during early development (Colman & Miller 2001). In the present study, morphine, at all doses studied, profoundly affected the breathing pattern, causing prolonged respiratory pauses. Respiratory rate was significantly lower indicating a depressant effect on PreBötzc. IBI was significantly prolonged and markedly variable resulting in respiratory instability. Our data is in accordance with previous studies showing that in rats at p10, fentanyl suppressed the frequency of respiration via μ -opioid receptors in PreBötzc (Greer et al 1995), and rats at p2–3 and p6–8 significantly decrease their frequency of breathing and increase their tidal volume via both μ_1 and μ_2 opioid receptor mediated activity (Colman & Miller 2001). In contrast to a previous report where breathing tracings were not included (Colman & Miller 2002a), morphine did not induce gasping in our animals. However, in support of our findings, morphine reduces anoxic gasping (Moore & Davis 1966) and thus we believe that morphine inhibits rhythm generation during normal breathing and gasping.

α_2 -adrenergic receptors are also present in the respiratory control centers in the pons, and adrenergic pathways have been identified in the nTS (Feldman & Moises 1988). Caudal administration of clonidine in neonates, specifically preterm infants, is associated with depression and apnea (Bouchut et al 2001). Our study is one of the first to explore the effect of clonidine on respiration in an in-vivo neonatal model. At the higher dose, clonidine significantly lowers the RR with increases in both T_i and IBI; however this effect does not result in prolonged respiratory pauses. The lack of effect of clonidine on respiratory parameters at the lower doses in immature models is particularly important and of translational significance.

CBT is tightly regulated in mammals. The pre-optic anterior hypothalamus is the site of the central thermoregulation. Autonomic defenses that maintain CBT include non-shivering thermogenesis, vasoconstriction and shivering (during hypothermia) or vasodilatation and sweating (during hyperthermia) (Nakamura & Morrison 2011). Neonates rely heavily on activation of non-shivering thermogenesis for maintenance of CBT. Thus, drugs that directly bind to receptors within the thermoregulatory circuitry or alter circulating levels of

catecholamines will modify CBT. Opioid and α_2 -adrenergic receptors are present on cells in the periphery and the pre-optic anterior hypothalamus during development, therefore, morphine and clonidine potentially affect CBT in neonates (Lin et al 1984, Mansour et al 1995).

In our model, morphine induces dose dependent hypothermia. These data are consistent with previous findings of opioid-induced hypothermia in neonatal rats between p2–14, with the greatest response occurring between p6–8 (Colman & Miller 2002b). The direction and magnitude of CBT responses caused by morphine varies widely depending on the species, dose and route of administration, degree of tolerance, restraint of the animal and ambient temperature (Adler et al 1988). We did not test all possible paradigms; however our results with IP injections are consistent with an exaggerated hypothermic response associated with systemic opioid administration in postoperative neonates (Okada et al 1998).

Thermoregulatory defenses causing hypothermia are also modified by α_2 -adrenoceptor agonists in adult animals. (Bhalla et al 2011). Clonidine is effective in the treatment of post-anesthetic shivering (Joris et al 1993). Clonidine lowers the set point for CBT and thereby reduces the threshold for vasoconstriction and shivering (Delaunay et al 1993). Adrenergic receptors are expressed on sympathetic neurons in the peripheral vascular bed and in the brown adipose tissue of the newborn rat (Skala et al 1988). There is a progressive increase in expression of α_2 -adrenergic receptors in the first 2–3 post natal weeks (Happe et al 2004). Here we show for the first time effects of clonidine on CBT in a pre-clinical neonatal model. Clonidine prevented the increase in CBT; it did not induce hypothermia but instead allowed the rat pups to maintain a stable temperature. Clonidine may have blocked sympathetic output to brown adipose tissue and decreased non-shivering thermogenesis. Since experiments were performed with one animal per chamber in which the environmental temperature was servo controlled, behavioral thermoregulatory defenses were not involved. Because both saline and clonidine animals moved about freely in the chamber it is doubtful that clonidine, at the dosages used, induced excessive sedation to account for the differences in CBT. We speculate that the increase in temperature observed in the saline treated animals by 60 minutes could be related to increase stress (Dallmann et al 2006, Ito et al 2006).

In addition to modifying respiratory and CBT responses, analgesic-sedatives also affects metabolism. Morphine, administered IP in neonatal rats results in a dose dependent reduction in VO_2 and VCO_2 by 90 min. In the immature rodent, metabolism and CBT at low and moderate doses of morphine were not directly correlated. Only the highest dose of morphine tested, lowered the metabolism resulting in hypothermia. This dissociation of metabolism and CBT at low and medium doses of morphine demonstrates that the change in metabolic rate at these doses does not precipitate a discernible fall in CBT. The effects of morphine on CBT, metabolism and respiration are similar to those that occur in hibernation in which endogenous opioids may play a role (Feng et al 2012).

Using whole body plethysmography in newborn rat pups at p7, our study demonstrated that clonidine reduces VO_2 and VCO_2 but only at the highest dose. Despite the effect on metabolism there was no effect on CBT at the highest dose. In contrast to the dose-dependent effect of morphine on VO_2 and VCO_2 , clonidine had a threshold effect at

400µg/kg. At this dose, clonidine could have a profound effect on sympathetic outflow caused by activation of presynaptic α_2 -adrenoceptors thereby decreasing the metabolic rate or blocking the metabolic effect on non-shivering thermogenesis in neonatal animals. While we expected that this would be associated with significant hypothermia, this was not found. These findings may be due to the developmental increase in α_2 -adrenergic receptor density in key brain regions after p7 (Happe et al 2004).

5. CONCLUSION

In newborn rat pups sedative-analgesics can have a profound and differential effect on respiratory network and CBT; morphine induces dose dependent hypothermia and prolonged respiratory pauses leading to respiratory instability while clonidine has minimal effect on the respiratory network at baseline and maintains CBT. Clonidine has a safe hemodynamic profile in term infants being treated for neonatal abstinence syndrome (Agthe et al 2009) and, in critically ill infants recovering from cardiovascular surgery (Pohl-Schickinger et al 2008). Thus, we speculate that clonidine may be a better alternative to morphine to prevent the discomfort associated with therapeutic hypothermia as it not only decreases shivering (De Witte & Sessler 2002) but also stabilizes CBT without causing respiratory depression.

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HIGHLIGHTS

- We compared two sedative-analgesics on breathing and temperature in neonatal rats
- Morphine causes profound respiratory depression and hypothermia
- Clonidine stabilizes body temperature, with only minimal effects on respiration

Figure 1. Representative breathing tracings of animals treated with saline, morphine and clonidine measured at baseline, 10 and 90 mins after drug administration

Both morphine (2 mg/kg) and clonidine (40 μ g/kg) decreased the RR when compared to animals treated with saline at similar time points. Morphine markedly affected the pattern of breathing with intermittent periods of prolonged pauses. Representative traces from 3 animals. *RR*, Respiratory Rate.

Figure 2. Bar graphs representing the RR

at (a) 10 minutes and (b) at 90 minutes after drug administration as a percent change from baseline. MOR₂, 10, 20 (mg/kg) significantly reduced RR by 45% (77 ± 17 breaths/min), 85% (28 ± 1.2 breaths/min) and 90% (23 ± 1.9 breaths/min), respectively at 90 mins. In contrast CLON₂₀₀, 400 (μ g/kg) reduced RR by only 35% (120 ± 9 breaths/min) and 45% (91 ± 2.6 breaths/min) respectively at 90 mins. $p < 0.05$, 2-way-ANOVA. (n=20/treatment group). RR, Respiratory Rate.

Figure 3. Bar graphs showing the effect of different dosages of treatment on (a) T_i and (b) IBI in p7 rat pups with MOR₂, 10 and CLON₄₀, 200. IBI was prolonged in MOR₂ pups by 90 mins and in MOR₁₀ pups at both 10 and 90 mins, $p < 0.001$, vs. saline pups. CLON₄₀ pups only transiently increased IBI at 10 mins, $p < 0.01$. T_i , inspiratory time; IBI, inter-breath interval.

Figure 4. Box and whisker plots showing the variability of T_i and IBI

at 10 and 90 mins after drug administration at low and moderate doses. IBI is more significantly affected than T_i . **(a) & (b)** T_i is prolonged from baseline in MOR₂ & MOR₁₀, $p < 0.01$ vs. baseline; only transiently prolonged in CLON₂₀₀ pups, $p < 0.01$ vs. baseline. **(c) & (d)** IBI is prolonged in morphine treated pups, the greatest variability seen in MOR₁₀ pups with a median of 2.5s and range of 0.3–11s. The values for MOR₂ (median 0.45, range 0.1–5s) CLON₄₀ (median 0.3, range 0.1–1.0s) and CLON₂₀₀ (median 0.4, range 0.2–1.5s) were much less variable. $p < 0.01$ vs. baseline. T_i , inspiratory time; IBI , inter-breath interval.

Figure 5. Breathing traces of p7 rat pup treated with morphine and p7 rat pup exposed to hypoxia

(a) Normal breathing pattern prior to morphine administration (b) 10 minutes after morphine administration demonstrating prolonged IBI with characteristic respiratory pauses (c) Eupneic breathing pattern prior to hypoxia and (d) gasping pattern (rapid inspiratory rise with a retarded expiratory phase) induced by hypoxia.

Figure 6. Line graphs showing the effects of MOR and CLON on CBT

in p7 rat pups at (a) higher doses – MOR_{10, 20}, CLON_{200, 400}. Saline controls increased their CBT in comparison to treatment, $p < 0.0001$, 2-way-ANOVA. Saline animals get progressively warmer by 2°C from baseline, $p < 0.0008$, 1-way-ANOVA. MOR₂₀ induced hypothermia compared to CLON_{200, 400}, $p < 0.005$, Bonferroni. (b) Bar graphs representing the change in CBT at 90 mins from baseline. At 90 mins saline controls are warmer than MOR_{2, 10, 20} & CLON_{40, 400}, $p < 0.05$, 2-way-ANOVA. MOR₂₀ animals are cooler by 3°C from baseline, $p < 0.0001$, 1-way ANOVA. (n=20/treatment group). CBT, Core Body Temperature.

Figure 7. Line graphs showing the effects of MOR and CLON on (a) VO₂ and (b) VCO₂ in p7 rat pups. MOR and CLON animals vs. saline controls have lower VO₂ and VCO₂, $p < 0.0001$, 2-way-ANOVA. Saline pups increase VO₂ overtime, $p < 0.01$. MOR₂₀ reduced VO₂ and VCO₂ in comparison to CLON₂₀₀, $p < 0.01$, Bonferroni. Bar graphs showing the comparison of (c) VO₂ and (d) VCO₂ in the treatment groups at 90 mins of the experiment. (c) VO₂ in CLON₄₀₀, MOR₁₀ and MOR₂₀ treated pups were 50%, 80% and 90% lower than saline controls respectively (d) VCO₂ in CLON₄₀₀, MOR₁₀ and MOR₂₀ pups were 45%, 65% and 80% lower than in saline pups respectively. $p < 0.001$, 2-way-ANOVA. (n=20/treatment group). VO₂, Oxygen consumption; VCO₂, carbon dioxide production.