Respiratory Physiology & Neurobiology 201 (2014) 93–100

Contents lists available at ScienceDirect

Respiratory Physiology & Neurobiology

journal homepage: www.elsevier.com/locate/resphysiol

Breathing and temperature control disrupted by morphine and stabilized by clonidine in neonatal rats

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ARTICLE INFO

Article history:
Accepted 29 June 2014
Available online 5 July 2014

Keywords:
Sedative-analgesics
Clonidine
Morphine
Respiratory depression
Hypothermia
Metabolism

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 min after intraperitoneal (IP) administration of morphine (2, 10 or 20 mg/kg), clonidine (40, 200 or 400 μg/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 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 main-stay 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). Alpha$_2$-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 (VO_2) in adults (Hurle et al., 1985), and causes either hypothermia, hyperthermia or both (Geller et al., 1983). In adults, clonidine, also depresses ventilation (McCrimmon and 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

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http://dx.doi.org/10.1016/j.resp.2014.06.015
1569-9048/

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 and 33 °C and three different dosages of morphine (2, 10 and 20 mg/kg) and clonidine (40, 200 and 400 μg/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 1–24 mg/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).

2.2.1. 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, unanaesthetized 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 and 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 (MOR_2 – 2 mg/kg, MOR_{10} – 10 mg/kg and MOR_{20} – 20 mg/kg) from Baxter Health Care Corp. (Deerfield, IL, USA) and clonidine hydrochloride ($CLON_{40}$ – 40 μg/kg, $CLON_{200}$ – 200 μg/kg and $CLON_{400}$ – 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 200 μl. 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 are expressed 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, Fig. 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 μg/kg) (Fig. 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 (Fig. 2). Compared to baseline, $MOR_{10,20}$ reduced RR by 85% (86 ± 1.9%) and 90% (89 ± 1.1%) at 10 min, respectively, with no additional change by 90 min (Fig. 2a and b). MOR_2 reduced RR by 45% (45 ± 12%) from baseline, which was only evident at 90 min (Fig. 2b). $CLON_{200,400}$ decreased RR by 30 (32 ± 3%) and 50% (50 ± 1.4%), respectively, at 90 min, in comparison to baseline. $CLON_{40}$ transiently decreased RR at 10 min (Figs. 1 and 2) but RR returned to baseline by 90 min. At baseline, all animals had a RR of approximately 182 ± 4.9 breaths/min. Ten minutes after drug administration, RR in MOR_{10} pups was 27 ± 3.9 breaths/min and in MOR_2 pups 106 ± 17.2 breaths/min, such that 85% and 40% of the time, MOR_{10} and MOR_2, animals, respectively were having respiratory pauses (prolonged IBI) (Fig. 5). At 90 min, in MOR_{10} pups, RR remained low at 28 ± 1.2 breaths/min and MOR_2 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. MOR_{10} caused a marked increase in IBI after 10 and 90 min of drug administration leading to prolonged respiratory pauses (Fig. 3). Compared