Pharmacology, Biochemistry and Behavior 168 (2018) 8–16

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

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

Age-dependent antinociception and behavioral inhibition by morphine

Alok Kumar Paul*, Nuri Gueven, Nikolas Dietis[1]

Division of Pharmacy, School of Medicine, University of Tasmania, Australia

ARTICLE INFO

Keywords:
Morphine
Motor behavior
Aging
Antinociception
Open-field
Locomotion

ABSTRACT

In current clinical practice, morphine is dosed in older patients based on patient-weight, with different calculations for adjustment. However, at present, neither clinical experience nor the literature offers a clear evidence base for the relationship between antinociception, behavioral effects and morphine administration in older patients. In this study, we compared the nociceptive response of 8 and 24 week old rats after subcutaneous administration of morphine *per body weight* and analyzed their behavior using an advanced multi-conditioning system. Residual morphine in all major tissues was determined. We observed prolonged morphine-induced antinociception in older rats compared to younger rats. Moreover, morphine significantly stimulated locomotor and rearing behavior 180 min after injection, which was significantly higher in the 8 week compared to 24 week old rats. Tissue analysis from animals extracted 240 min post-injection revealed a significantly higher concentration of residual morphine in the brains of older versus younger animals when standardized on tissue weight. However, this effect was not observed when residual morphine was standardized on protein content. Collectively, our data suggest that in older rats morphine exhibits higher antinociception and increased behavioral inhibition compared to younger animals. This effect is likely due to a significantly higher accumulation of morphine in the brain of older animals.

1. Introduction

The proportion and number of older people is increasing globally with an expected 20% of the total population above 60 years of age by 2050 (WHO, 2015). These individuals experience pain comparatively more than younger people, which affects their daily activities and total quality of life. Noticeably, due to the high prevalence of pain, individuals over 60 years of age are the highest users of analgesics and especially of opioids (McLachlan et al., 2011). Therefore, the effective management and safe use of opioids is particularly important for this cohort. Morphine, one of the most frequently used opiates worldwide, is considered a high-risk medication due to its narrow therapeutic index and a plethora of neuropsychological and behavioral effects (Lloret Linares et al., 2009; Bilkei-Gorzo et al., 2010; Srinivas, 2013). Therefore, different calculative dose-adjustments are often clinically used to ensure drug safety, but do not always provide effective pain relief (Aubrun and Marmion, 2007; Mercadante and Arcuri, 2007; Yen et al., 2010; Coldrey et al., 2011; Falzone et al., 2013).

Although, the prevalence of pain in the aging population is high, these patients are often not properly assessed regarding their pain relief (Hall-Lord et al., 1998; Werner et al., 1998; Rundshagen et al., 1999; Herr and Garand, 2001; Paulson et al., 2014), which severely reduces treatment efficacy (Bernabei et al., 1998; Varner, 2012; MacSorley et al., 2014). A striking lack of data for the aging population regarding the effects of opioid dosing on patient behavior is especially evident for patients with cognitive impairment (Shega et al., 2006; Corbett et al., 2012; Brorson et al., 2014; Romem et al., 2015). Furthermore, compared to younger patients, aged patients show a higher incidence of adverse events, which are especially associated with long-term opioid treatment (Falzone et al., 2013; Schuler and Griessinger, 2015). Therefore, the selection of effective and safe morphine doses in aged individuals is one of the most frequently faced challenges in the clinic. The difficulty to achieve adequate pain relief and at the same time to avert the manifestation of side effects in aged patients, has fueled concerns that factors such as altered drug pharmacokinetics, metabolism or behavioral changes could contribute to this challenge (Aubrun and Marmion, 2007; Mercadante and Arcuri, 2007; Coldrey et al., 2011).

Therefore, there is an urgent need to identify a possible correlation between the antinociceptive and behavioral effects of morphine in aged individuals. At present, a limited number of studies suggest a physiological and/or molecular basis for the differences observed in opioid pharmacology when comparing aged and younger individuals (Tucker et al., 1989; Simon et al., 2015). However, the evident lack of

* Corresponding author at: Division of Pharmacy, School of Medicine, University of Tasmania, Private Bag 26, Hobart, TAS 7001, Australia.
E-mail address: alok.paul@utas.edu.au (A.K. Paul).
[1] Current address: Medical School, University of Cyprus, Nicosia, Cyprus.

https://doi.org/10.1016/j.pbb.2018.03.003
Received 16 August 2017; Received in revised form 2 February 2018; Accepted 12 March 2018
Available online 13 March 2018
0091-3057/

established knowledge regarding the behavioral effects of opioids in aged subjects and the potential differences compared to young individuals hinders the legitimate and appropriate use of opioids in aged individuals.

In this study, we connected the antinociceptive and behavioral activities of morphine with residual morphine concentrations in post-mortem tissues of test animals, to shed some light on the age-dependent pharmacokinetic and behavioral differences of morphine administration.

2. Materials and methods

2.1. Materials

Morphine sulfate solution for injection (30 mg/ml) was purchased from Hameln Pharmaceuticals GmbH, Germany. The drug was kept at room temperature in a secured safe in accordance with Australian regulations around schedule 8 drugs. For the low dose group (5 mg/kg), morphine sulfate was diluted to 15 mg/ml with sterile 0.9% sodium chloride solution immediately prior to subcutaneous (*s.c.*) injection. The injection volumes for both 5 and 10 mg/kg morphine groups were the same according to volume/weight and within each group differed by not > 8% when adjusted for individual animal weights. F10SC veterinary disinfectant solution (Health and Hygiene Pty Ltd, Florida Hills, South Africa) was used for cleaning and hygiene purpose as a diluted solution (1:250 in water).

2.2. Animals

Male Sprague Dawley (SD) rats (total 20; 4 × n = 5) were used in this study. Half of those (n = 10) aged for 24 weeks using normal diet (6% crude fat, Barastoc rodent cubes, Ridley Corporation, Melbourne, Australia) and water, while the other animals (n = 10) aged for only 8 weeks on the same diet. During the experimental phase all animals were single-housed under standard laboratory conditions and kept on an automated 12:12 h day/night cycle (lights on at 7:00 am). The treated animals were single-housed after administration of morphine and throughout the experiments in order to facilitate behavioral health-monitoring and avert the manifestation of potential morphine-induced behavioral aggression towards cage-mates. Animals were handled for 5–6 days before the experiments were conducted. On the morning of behavioral tests, animals were transported to the testing room in their home case and acclimatized to the test environment for 1 h. All procedures and animal handling were performed according to the guidelines of the University of Tasmania Animal Ethics Committee (approval no. A0013864) and *The Australian Code for the Care and Use of Animals for Scientific Purposes* (NHMRC, 2013). Animals of each group (8 and 24 weeks of age) were divided into two subgroups using a completely randomized design as previously described (Festing and Altman, 2002). Each group received a different dose of morphine sulfate solution (5 mg/kg or 10 mg/kg) as single s.c. injection between the left thigh and the spinal cord. Subcutaneous administration was previously shown to be an effective and quick route for morphine administration (Stuart-Harris et al., 2000), causing minimal discomfort to the animals. The two different doses of morphine were selected, based on previous data from our group regarding the connection between morphine loading-dose and antinociception (Paul et al., 2017). The weight of all animals was recorded prior to the administration of morphine. The BMI of rats was calculated based on the formula: $BMI = body\ weight\ (g) / body\ length^2\ (cm^2)$ as described previously (Engelbregt et al., 2001; Novelli et al., 2007). The animals were tested side-by-side by the same operator in the same testing environment, but extensive cleaning and hygiene procedures were undertaken to ensure that the younger animals were not exposed to the scent of the older animals and vice versa. A schematic diagram represents the different time-points before and after morphine treatment of 8 and 24 week old animals (Fig. 4 in Paul et al. (2018)).

2.3. Nociception measurements

Nociception was determined independently by tail-flick and hot-plate assays performed in random sequence, separated by a 1 min interval between the two assays, using specialized apparatuses (Ugo Basile, Comerio, Italy). Maximum exposure of the animals to the nociceptive thermal stimuli (cut-off time) was 15 s for the tail-flick and 30 s for the hot-plate assay, as previously described (Heilborn et al., 2007; Khroyan et al., 2011; Duan et al., 2014; Paul et al., 2017). The infrared intensity of the tail-flick photocell was set at 30, whereas the plate temperature of the hot-plate was set at 54 ± 0.5 °C. All rats were tested immediately prior to morphine administration (basal measurement) and at 15, 30, 60, 120, 180 and 240 min post-administration in both assays. The maximum possible effect (MPE) was defined as % MPE = 100 × [(test latency − baseline latency) / (cut-off time − baseline latency)] as previously described (Harris and Pierson, 1964). Animals treated with 3 mg/kg morphine showed similar maximum antinociception (both MPE% and latencies) compared to 5 mg/kg treated animals 30 min post-injection in both antinociception assays (data not shown). Therefore, an EC_{50} dose could not be determined between 3 and 10 mg/kg morphine.

Nociception experiments were conducted blindly and results were recorded by averaging three independent measurements for each time-point with a 1 min difference between measurements to minimize 'handling' effects. No differences in basal nociceptive thresholds were observed in both antinociception assays between 8 and 24 week old rats, which supports two previous studies, that also observed no age-related differences in basal antinociception (Jourdan et al., 2000; Jourdan et al., 2002). In addition, no differences in antinociceptive latencies over a period of 2 h were detected (data not shown). Similarly, over a period of 2 weeks of repeated testing, basal levels of antinociceptive latencies also remained unchanged (Suppl. Fig. S4 in Paul et al. (2017)), which both indicate that 'testing fatigue' did not affect the experimental results of this study.

2.4. Behavioral measurements

The behavioral testing used six different activity parameters (total distance travelled, rearing time, ratio of presence in periphery versus center, clockwise rotation, anti-clockwise rotation and moving time). Behavior was tested in an open-field arena in a Multi-Conditioning System (MCS) (TSE GmbH, Homburg, Germany) 2 min after nociception-testing at 0 min (pre), 30 and 180 min after administration of morphine over a period of 5 min. Measurements in open field arena over a period of 5 min are commonly used (Rex et al., 1998; Prut and Belzung, 2003; Sestakova et al., 2013; Hollais et al., 2014) and allowed the concurrent measurements of antinociception in the same group of animals in this study. The MCS platform included an internal noise/light/temperature insulation system and a 3D infrared-beam frame that provided fast and accurate animal movement detection (100 Hz), combined with a high-resolution video monitoring and automated movement tracking system. Quantification and visualization of the MCS data were processed by integrated system software (TSE ActiMot). The open-field arena was thoroughly cleaned between each animal using paper towels soaked in diluted F10 solution. Background white noise (20 dB) was used during all experiments to cancel out environmental sounds.

2.5. Tissue collection

Immediately after testing antinociception at 240 min post-administration of morphine, animals were anesthetized with 5% (w/v) isoflurane in oxygen at a flow rate of 1 l/min, until loss of consciousness was observed (usually 5–7 min) and the animals were decapitated. Blood was collected from the decapitated body by gravity flow using 15 ml centrifuge tubes (Corning Centristar) and immediately