Chronic administration of morphine using mini-osmotic pumps affects spatial memory in the male rat

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A B S T R A C T

The use of opioid analgesics to treat non-cancer pain has increased over the years. Many chronic pain patients suffer from numerous adverse effects, such as reduced quality of life, development of dependence, and cognitive impairments. Cognitive processes are regulated by several systems, one of which involves growth hormone (GH) and its secondary mediator insulin-like growth factor-1 (IGF-1), but also glutamatergic transmission, including receptors such as the N-methyl-D-aspartate (NMDA)-receptor complex. In the laboratory, repeated injections are commonly used to establish animal models of long-term or chronic drug exposure. However, in the present study, we aimed to mimic a more human dose regimen using constant drug delivery provided by mini-osmotic pumps implanted subcutaneously in male Sprague Dawley rats. After developing opioid tolerance the cognitive function of rats was studied. Spatial learning and memory capabilities were evaluated using the rat Morris water maze (MWM). Moreover, gene expression related to the GH/IGF-1-axis and the NMDA-receptor system was analyzed using quantitative PCR (qPCR) and plasma levels of IGF-1 were assessed using the ELISA technique. Our results demonstrate that rats exposed to morphine for 27 days display memory impairments in the MWM. Furthermore, gene expression related to the GH/IGF-1-axis and the NMDA-receptor system was analyzed using quantitative PCR (qPCR) and plasma levels of IGF-1 were assessed using the ELISA technique. The animal model used in this study provides a simple and suitable way to investigate the behavioral and neurochemical effects of chronic opioid treatment similar to the exposure seen in human pain patients.

1. Introduction

Opioids, including morphine, are well known pharmaceutical drugs that are used worldwide to treat moderate and severe pain. To date, opioid drugs are the most efficient analgesics available, but they are also associated with several adverse effects, for example respiratory depression and the risk of addiction. Additionally, there is evidence for a link between substance use disorders and altered cognitive behavior (for review see Gould, 2010, Nyberg, 2012). In animal models, administration of opioid drugs impairs performance in several types of learning and memory tasks (Dougherty et al., 1996; Sala et al., 1994; Spain and Newsom, 1991; Tramullas et al., 2007). In humans, cognitive dysfunction has been reported in both non-cancer patients receiving long-term opioid treatment (Sjögren et al., 2000) as well as in low back pain patients (Schielenwolff et al., 2014). Furthermore, chronic pain patients treated daily with strong opioids display hormonal alterations and reduced quality of life, including physical, social and emotional functioning (Rhodin et al., 2010). Taken together, these animal and human studies indicate that opioids may alter and modulate cognitive processes.

Neuronal alterations observed in the central nervous system (CNS) following opioid exposure could be a plausible explanation to the psychological impairments seen in these individuals (Boronat et al., 2001; Eisch et al., 2000; Hu et al., 2002). The neurobiological mechanisms underlying cognitive processes are complex, but the induction of long term potentiation (LTP) involving the N-methyl-D-aspartate (NMDA) receptor complex is considered to be crucial for memory formation (Huang et al., 2001). For instance, transgenic mice with an increased expression of the GluN2b receptor subunit exhibit improved performance in the Morris water maze (MWM) and the Novel Object Recognition (NOR) task, indicating that this subunit is essential for long-term memory (Tang et al., 1999). In our previous studies, we have reported effects of both acute and chronic morphine administration on different NMDA receptor subunits (Johansson et al., 2010; Le Greves...
et al., 1998). Moreover, NMDA-receptors are known to be important for drug-induced associative memories (Hu et al., 2002). Another system that has been implicated in neuroprotection and cognitive functioning is the somatotrophic axis i.e. GH/IGF-1-system (for review see Nyberg, 2009; Nyberg and Hallberg, 2013). Primarily known as a mitogenic polypeptide, growth hormone (GH) has in several studies, together with insulin-like growth-factor 1 (IGF-1) and insulin-like growth factor-2 (IGF-2), been recognized for its neuroprotective and cognitive enhancing properties in the CNS (Chen et al., 2011; Nyberg and Hallberg, 2013; Åberg et al., 2006).

The aim of the present study was to a) investigate the behavioral effects of chronic morphine delivered in mini-osmotic pumps, primarily on learning and memory function, but also on the development of opioid tolerance; b) evaluate the biochemical effects of continuous morphine treatment, more specifically to study mRNA expression of entities related to the somatotrophic axis and the NMDA receptor system in brain regions essential for cognitive function; and finally c) to examine the plasma levels of IGF-1 using enzyme-linked immunosorbsent assay (ELISA).

2. Materials and methods

2.1. Animals

Male Sprague Dawley rats (Taconic, Denmark), eight weeks old at arrival, were used in the experiment. The animals were housed two per cage and kept under a reversed 12 h dark/light cycle with lights on at 18:00 h. Standardized housing conditions were used (i.e. 20–24 °C and a humidity of 45–65%) and behavioral testing was performed during the dark phase. Rats were provided with food and water ad libitum, monitored daily, and weighed weekly. After two weeks of acclimatization, rats were randomly divided in two treatment groups, with eight individuals per group. Osmotic mini-pumps containing saline or morphine hydrochloride were implanted under the skin (for details see the section below). Following decapsulation, trunk blood was collected in tubes containing 500 μL of ice-cold 1% (w/v) EDTA in 0.9% (w/v) NaCl. The tubes were centrifuged for 10 min, at 3000 rpm at 4 °C, and the plasma fractions were subsequently collected. Selected brain regions, including the frontal cortex, hippocampus and caudate putamen (Paxinos and Watson, 1997) were dissected using a rat brain matrix (Activational System, Warren, MI, USA), and rapidly frozen on dry ice. Both plasma and brain tissue were stored at −80 °C until ready for biochemical analysis. Animal experiments followed the guidelines of the Swedish legislation on animal experimentation and were approved by the Uppsala Animal Ethical Committee.

2.2. Filling of osmotic pumps

Morphine hydrochloride 17.5 mg/kg/day (Apoteket AB, Stockholm, Sweden) was administered continuously using ALZET® osmotic pumps model 2ML4, 2.5 μL per hour for a maximum of 28 days (Alzet, Cupertino, CA, USA). The dose was chosen based on previous studies in the field (Johansson et al., 2010; Pu et al., 2002). In order to increase the solubility, morphine hydrochloride was dissolved in saline and 2% (v/v) dimethyl sulfoxide (DMSO). Both the saline solution and the morphine solution were filtered through a sterile 0.2 μm filter before the procedure was initiated. Further, filling and preparation of mini-osmotic pumps for implantation was performed according to the instructions provided by the manufacturer.

2.3. Implantation of osmotic pumps

Animals were anesthetized with isoflurane (Abbot Scandinavia, Solna, Sweden) 4% (v/v) for induction and 3% (v/v) for maintenance during surgery. Oculentum Simplex eye gel (APL, Kungens Kurva, Sweden) was applied to prevent the eyes from dehydration. An incision in the skin was made in the lumbar region and the skin was opened by means of blunt dissection to allow implantation of the pumps. The mini-osmotic pumps, two per rat, were placed subcutaneously and the skin was sutured with absorbable thread. Rats were allowed to recover from anesthesia, for approximately 1 h, in a separate cage before being returned to their home cage.

2.4. Tail flick

To observe the development of opioid tolerance a standard analgesic assay, the tail flick test (Model 33 Tail Flick Analgesia Meter, IITC, Life Science, USA), was used at five different time points during the experimental period (for details see the experimental outline presented in Fig. 1). Each animal was tested twice and the mean value of the measurements was referred to as the tail flick latency. The cut-off latency was set at 10 s to avoid tissue damage. The latency value from the tail flick test assessed before morphine treatment commenced (i.e. on experimental day 0) was defined as the baseline.

2.5. Morris water maze (MWM)

The MWM is a well-established behavioral test used to assess spatial learning and memory in rodents (Morris et al., 1982). The MWM protocol used in the present study was adapted from our previous study (Grönbladh et al., 2013). The equipment consisted of a circular pool, with a diameter of 160 cm, that was divided into four different quadrants; north west (NW), north east (NE), south east (SE), and south west (SW). A transparent platform, referred to as the hidden platform, was placed approximately 1.5 cm underneath the water surface in the SW quadrant (the target quadrant). The pool was filled with tap water with the temperature controlled at 22 ± 1 °C. Behavioral testing was assessed at the end of the experimental period (for details see the experimental outline presented in Fig. 1). The MWM-test was divided into a training session of five consecutive days (acquisition phase) followed by a memory test (probe trial) initiated 72 h after the last trial in the maze. At the start, the experimenter placed the rat in different quadrants randomized for each trial. In the acquisition phase rats were allowed four trials each day and in case the rat did not find the platform
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