Available online at www.sciencedirect.com
ScienceDirect
Life Sciences 80 (2007) 1915–1920
Life Sciences
www.elsevier.com/locate/lifescie

Chronic morphine exposure increases the proportion of on-cells in the rostral ventromedial medulla in rats

Ian D. Meng [a,*], Ichiro Harasawa [b]

[a] Department of Physiology, College of Osteopathic Medicine, University of New England, 11 Hills Beach Rd. Biddeford, ME 04005, USA
[b] Department of Physiology, University of California, San Francisco, 513 Parnassus Ave, San Francisco, CA 94143, USA

Received 21 August 2006; accepted 19 February 2007

Abstract

Chronic opiate exposure produces tolerance and hypersensitivity to mechanical and thermal stimulation that involves increased pain facilitation from the rostral ventromedial medulla (RVM). The aim of the present study was to determine the effect of sustained systemic morphine exposure on RVM neurons. Three cell types in the RVM have been described: on-cells, off-cells and neutral cells. The activity of on-cells increases in response to noxious stimulation, whereas the activity of off-cells decreases following noxious stimulation. Neutral cells remain relatively unaffected. In lightly anesthetized rats, systematic exploration throughout the RVM using single-unit extracellular recordings was used to examine both the relative proportion and the neuronal properties of the different cell classes in chronic morphine and placebo treated animals. Seven days after implanting either morphine (150 mg, s.c.) or placebo pellets a total of four electrode penetrations through the RVM were made in each animal at identical coordinates along midline. Neuronal responses related to radiant heat-evoked paw withdrawals were recorded. When compared to placebo treated rats, chronic morphine increased the number of on-cells and decreased the number of neutral cells, while the number of off-cells remained unchanged. Chronic morphine exposure had no effect on the spontaneous or heat-evoked discharges in on-, off-, or neutral cells. These results indicate that chronic morphine may sensitize a subpopulation of RVM neurons to noxious stimulation, which would be expected to increase descending facilitation and promote tolerance and chronic morphine-induced paradoxical pain.

Keywords: Nucleus raphe magnus; Morphine; Pain

Introduction

Prolonged exposure to opiates can produce tolerance to its analgesic effects and, in some cases, induce paradoxical pain (Compton et al., 2001; Doverty et al., 2001; King et al., 2005b; Sjogren et al., 1994). In animal models, chronic administration of either systemic or spinal morphine produces tolerance and hypersensitivity to both mechanical and thermal stimulation (Gardell et al., 2002; Laulin et al., 1999; Mao et al., 1994; Vanderah et al., 2001a,b). Several neuroplastic changes involving the up-regulation of pronociceptive systems have been described that could contribute to chronic morphine-induced hypersensitivity. Sustained morphine exposure increases calcitonin gene-related peptide (CGRP) content in dorsal root ganglion neurons both in vivo and in cell cultures (Belanger et al., 2002; Gardell et al., 2002; Menard et al., 1996) and potentiates capsaicin-evoked release of CGRP and substance P from spinal cord tissue (Gardell et al., 2002; King et al., 2005a). Additionally, sustained morphine increases expression of spinal dynorphin, a peptide with pronociceptive effects possibly mediated through NMDA receptors, and down-regulates glutamate transporters in spinal cord tissue (Gardell et al., 2002; Mao et al., 2002; Vanderah et al., 2000, 2001a).

Many of these chronic morphine-induced events require input from the rostral ventromedial medulla (RVM). The RVM, which includes neurons in the midline nucleus raphe magnus and the adjacent reticular formation, modulates nociception through both indirect and, via the dorsolateral funiculus (DLF), direct projections to the spinal and medullary dorsal horn (Fields et al., 1991). Lidocaine microinjections into the RVM reverse mechanical and thermal hypersensitivity and attenuate

* Corresponding author. Tel.: +1 207 602 2195; fax: +1 207 294 5931.
E-mail address: imeng@une.edu (I.D. Meng).

0024-3205/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.lfs.2007.02.022

tolerance produced by prolonged administration of morphine (Vanderah et al., 2001b). Furthermore, lesions of the DLF prevent sustained morphine-induced hypersensitivity as well as the increases in evoked CGRP release and dynorphin content from spinal cord tissue (Gardell et al., 2002). These data suggest that sustained morphine exposure increases the activity of RVM neurons involved in facilitating nociceptive signals at the level of the dorsal horn.

Noxious stimulation produces three distinct patterns of activity in RVM neurons that are correlated with withdrawal reflexes (Fields et al., 1983a, 1991). On-cell activity increases with the occurrence of a nociceptive reflex, off-cell activity decreases with the nociceptive reflex and neutral cells show little or no consistent change in firing rate related to withdrawal responses. Strong evidence implicates on-cell activity in facilitating and off-cell activity in inhibiting nociceptive transmission (Bederson et al., 1990; Fields et al., 1983b; Foo and Mason, 2003; Heinricher et al., 2004, 1994; Heinricher and Neubert, 2004; Kaplan and Fields, 1991; Kincaid et al., 2006; Meng et al., 1998; Mitchell et al., 1998; Neubert et al., 2004). The present study examined the effect of sustained systemic administration of morphine on the physiological characteristics and relative proportions of each RVM neuronal cell type.

Materials and methods

Studies were conducted using male Sprague–Dawley rats weighing 200–250 g at the time of recording. Animals were housed two per cage in a room maintained with an alternating 12 h light–dark cycle. The present study was approved by the Committee on Animal Research at University of California San Francisco and the University of New England, and animals were treated according to the policies and recommendations of the NIH guidelines for the handling and use of laboratory animals.

Surgery

Seven days prior to recording, rats were implanted subcutaneously with two pellets containing either morphine sulfate (75 mg pellets) or placebo under isoflurane anesthesia. On the day of recording, rats were injected with sodium pentobarbital (60–70 mg/kg, i.p.) and a catheter inserted into the external jugular vein for administration of anesthetics. The femoral artery was cannulated for measuring blood pressure. Mean arterial pressure (MAP), heart rate and respiratory rate were recorded prior to each electrode penetration during the experiment. Body temperature was maintained at 37 °C with a hot water circulating heating pad. After placing the rat into a stereotaxic holder, a hole was drilled in the interparietal bone for insertion of an electrode into the medulla. Anesthesia was maintained with a constant infusion of sodium pentobarbital (1–10 mg/kg/h) at a level to prevent signs of discomfort and allow stable paw withdrawals with an average latency between 3.0 and 4.0 s using a feedback controlled projector lamp. Movement of the hind paw was recorded using a mechanical transducer, and the rate of rise in temperature and peak holding temperature was identical in all experiments. Cell recordings were initiated at least 45 min after starting the infusion of sodium pentobarbital.

Electrophysiological recordings

A total of four electrode penetrations at 0.4 mm intervals were made in each animal in similar coordinates along midline, from 1.2–2.4 mm caudal to interaural zero. Extracellular single-unit activity was recorded from 7.5 to 9.0 mm below the surface of the cerebellum using tungsten electrodes (3 MΩ, FHC, Bowdoinham, ME) using methods as previously described (Harasawa et al., 2000; Meng and Johansen, 2004; Meng et al., 2005). Briefly, the amplified, filtered signal was passed through a window discriminator and monitored with digital and storage oscilloscopes. Discriminated units triggered a digital oscilloscope to confirm constant spike shape and amplitude. Data were acquired and analyzed off-line using LabView (National Instruments, Austin, TX).

Neurons from the RVM were systematically sampled to allow for comparisons between treatment groups. Periodic light brushing and pressure of the hind paw as well as heat-evoked paw withdrawals were used to search for neurons. Ample time was given between stimuli in order to allow ongoing activity of cells to return. In order to minimize the possibility of sample bias, every neuron that could be isolated with a signal to noise ratio of at least 3:1 was characterized with noxious thermal stimulation of the hindpaw. At least two paw withdrawal trials were performed at 3–5 min intervals, and baseline and heat-evoked changes in activity were analyzed.

On-, off-, and neutral cells were categorized according to their pattern of neuronal activity as it related to the paw withdrawal reflex (Heinricher et al., 2004; Neubert et al., 2004). On-cells were identified by a sudden burst of activity while off-cells demonstrated a pause in activity that occurred at the time of withdrawal. Neutral cells demonstrated continuous firing that was not affected by the noxious thermal stimulus. The lack of response to thermal stimulation was also confirmed with noxious mechanical stimulation applied to the tail and hindpaw. The paw withdrawal reflex was used instead of the tail flick because previous studies have demonstrated behavioral hypersensitivity using paw stimulation (Gardell et al., 2002; King et al., 2005a; Vanderah et al., 2001b; Xie et al., 2005).

In separate experiments, the presence of tolerance and chronic morphine-induced hypersensitivity was examined following morphine pellet implantation. Paw withdrawal latencies were measured using radiant heat delivered from a Hargreaves apparatus immediately prior to and 7 days following pellet implantation.

Data analysis

Analysis was performed on the ongoing (spontaneous) activity and heat-evoked changes in discharge of all cell types. Ongoing activity was calculated as the average frequency over a 60 s epoch immediately prior to the heat onset. Paw withdrawal related activity was calculated as the average frequency of activity over a 10 s period following the heat onset. In addition,