Brain Research Bulletin 137 (2018) 178–186

Contents lists available at ScienceDirect

Brain Research Bulletin

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

Research report

Chronic adolescent morphine exposure alters the responses of lateral paragigantocellular neurons to acute morphine administration in adulthood

Hamed Salmanzadeh, Hossein Azizi*, S. Mohammad Ahmadi Soleimani, Narges Pachenari, Saeed Semnanian

Department of Physiology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran

ARTICLE INFO

Keywords:
Adolescence
Morphine
Extracellular in vivo single unit recording
LPGi nucleus
Long-lasting effect

ABSTRACT

Accumulating evidence support the growing non-medical use of morphine during adolescence. Despite this concern which has recently been addressed in some studies, cellular mechanisms underlying the long-term neurobiological and behavioral effects of opiate exposure during this critical period have still remained largely unexplored. Several reports have proposed that subtle long-lasting neurobiological alterations might be triggered by exposure to opiate derivatives or drugs of abuse particularly when this occurs during a critical phase of brain maturation such as adolescence. The present study was designed to investigate how chronic adolescent morphine exposure could affect the responsiveness of lateral paragigantocellular (LPGi) neurons to acute morphine administration in adult rats. Male Wistar rats received chronic escalating morphine or saline during adolescence (30–39d) for 10 days. During adulthood (65d), the extracellular unit activities of LPGi neurons were recorded in urethane-anesthetized animals. Results indicated that adolescent morphine treatment enhances the baseline activity of LPGi neurons. In addition, morphine-induced inhibition of spontaneous discharge rate was potentiated in adult rats received morphine during adolescence. However, this pretreatment did not affect the extent of morphine excitatory effect, onset or peak of cellular response and regularity of unit discharge in LPGi neurons. Our study supports the hypothesis that adolescent morphine exposure induces long-lasting neurophysiological alterations in brain regions known to play a role in mediating opiate effects. This finding sheds light on the possible effect of opiate pre-exposure on addiction susceptibility in future.

1. Introduction

Opioid drugs are among the most widely abused pharmaceuticals throughout the world. This common abuse is usually facilitated because opiate derivatives are generally considered by their users as relatively harmless (Primavera and Pascal, 1986). Similar to other drugs of abuse, the use of opioid analgesics is particularly problematic in adolescents and young adults (Koek, 2014). This is mainly because long-term opioid exposure during adolescence has been shown to affect addiction susceptibility during adulthood (White et al., 2008). Adolescence is known as a critical developmental period characterized by distinct neurobiological processes (Spear, 2000). The immature central nervous system undergoes a series of physiological and anatomical alterations during the postnatal period prior to the final maturation of brain organization such as transmitters, receptors and pathways (Rahman and Dickenson, 1999). Human epidemiological evidence suggests that adolescents with the experience of drug abuse are more susceptible to resume drug taking in adulthood (Robins and Przybeck, 1985; Simoni-Wastila and Yang, 2006). In this regard, a recent study by this group (Salmanzadeh et al., 2017) indicated that repeated morphine administration in adolescent rats facilitates the development of morphine dependence and tolerance in adulthood. In addition, in another study, it was reported that rats exposed to morphine during adolescence display an enhanced risk of relapse to drug-seeking behaviors in adulthood as the result of long-term changes in microglial functions in NAc (Schwarz and Bilbo, 2013). It is also worthwhile to mention that rats treated with morphine during adolescence exhibit a higher locomotor response to morphine during adulthood. This supports the idea that adolescent's opioid system is more sensitive to locomotor sensitization (White et al., 2008).

Lateral paragigantocellularis (LPGi) nucleus, located in the rostral ventrolateral medulla (RVLM), has been shown to play a critical role in the development of opiate tolerance and dependence (Ahmadi-Soleimani et al., 2017). Although, some evidence has previously reported that adolescent exposure to various drugs of abuse could result in prolonged alterations in the neuronal function of different brain regions, the effect of adolescent morphine exposure on LPGi neuronal

* Corresponding author.
E-mail address: azizih@modares.ac.ir (H. Azizi).

https://doi.org/10.1016/j.brainresbull.2017.12.007
Received 4 October 2017; Received in revised form 20 November 2017; Accepted 7 December 2017
Available online 12 December 2017
0361-9230/

response during adulthood has not been addressed in the literature. Our previous behavioral findings revealed that adolescent morphine exposure could facilitate opioid-induced effects in adulthood (Salmanzadeh et al., 2017). Thus, the present study was designed to investigate whether chronic morphine administration in adolescent rats affects the LPGi neuronal response to acute morphine exposure in adulthood.

2. Materials and methods

2.1. Animals

A total number of 48 male Wistar rats (Razi Institute, Tehran, Iran) were used in this study. 23 days old rats were kept four per cage in a colony room with a regular controlled temperature, 12-h light/dark cycle (lights on at 7:00 a.m.) and free access to food and water. This study was performed in accordance with the ethical guidelines of the Faculty of Medical Sciences Ethics Committee, Tarbiat Modares University, which are based on NIH Guide for the Care and Use of Laboratory Animals.

2.2. Drugs

Morphine sulfate (Temad, Tehran, Iran) was dissolved in 0.9% injectable saline. For each experiment, the freshly prepared solution was injected intraperitoneally (i.p.).

2.3. Adolescent morphine exposure

Adolescent rats (30 days old) were randomly divided into two experimental groups for receiving morphine or saline (morphine- and saline-treated groups, respectively). On day 1, rats were injected 2.5 mg/kg morphine sulfate or saline (2 ml/kg) subcutaneously (s.c.) twice daily (8:00 a.m. and 5:00 p.m.). On each subsequent day, the dose of morphine was escalated by 2.5 mg/kg till the day 10 in which animals received the dose of 25 mg/kg (Byrnes et al., 2011; Salmanzadeh et al., 2017)(Fig. 1).

2.4. Surgical procedures and extracellular single-unit recording

According to the protocols used in our previous studies (Ghaemi-Jandabi et al., 2014), adult rats (65 days old) were anesthetized with i.p. injection of urethane (1.2 g/kg). Additional (maintenance) doses (0.15 g/kg, i.p.) were administered every 1 h to achieve a deep and stable level of anesthesia. Animals were first tracheostomized to minimize the noises generated by respiratory movements and then were secured in a stereotaxic apparatus. The animal head was fixed in the stereotaxic frame (Narishige, Japan) by two blunt-ended ear bars and the incisor bar was set at −3.3 mm. A small hole (2 mm in diameter) was carefully drilled in the skull right above LPGi nucleus (12 mm caudal to bregma, and 1.5 mm lateral to midline). The coordinates were determined according to the atlas of Paxinos and Watson (Paxinos and Watson, 1998). The extracellular electrical activity of only one neuron was recorded from each rat. LPGi neuronal activity was recorded using glass micropipettes (3–12 MΩ resistance) filled with 2% pontamine sky blue in 0.5 M sodium acetate. Micropipettes were stereotaxically lowered down towards the LPGi region (approximately 10.2–10.8 mm ventral to skull surface).

When lowering the recording electrode, a typical bursty pattern of neuronal activity (belonging to the Bötzinger or pre-Bötzinger nuclei) served as an electrophysiological landmark (Fig. 2A) (Sirieix et al., 2012). This specific pattern (Fig. 2A) was strongly in phase with the animal's respiratory movements. The neuronal activities recorded after the last respiratory signals were considered as the LPGi neurons (Sirieix et al., 2012)(Fig. 2B).

Extracellular neuronal activity was amplified (×1000) with an AC differential amplifier (DAM 80, WPI, USA). Signals were then filtered (0.3–3 kHz bandpass) and continuously displayed on an oscilloscope (Hitachi, Japan). Recorded spikes were also monitored simultaneously using an audio analyzer set (Fredrick Haer, USA). Recorded signals were then digitized by a commercial analog to digital data acquisition system (PowerLab 4/30, ADInstruments Pty Ltd., Australia). Finally, the obtained data were analyzed by Lab Chart 7 software with the Spike Histogram Module (ADInstruments) and spikes were displayed as rate histogram (1 min bin size). Morphine (2 mg/kg) was dissolved in 2 ml saline 0.9% and injected (i.p.) after 15 min of baseline recording from LPGi neurons. Any increase or decrease in spontaneous discharge rate which calculated by mean ± 2 × standard deviation (SD), was considered as an excitatory or inhibitory effect. Also, the coefficient of variation (CV) of interspike intervals (ISIs) (SD/mean ISIs × 100), used as a quantitative measure of the regularity of unit discharge, was calculated for each LPGi neuronal response to morphine (excitatory, inhibitory and no effect) in both experimental groups.

2.5. Data analysis

Statistical analyses were done by GraphPad Prism version 6.01 for Windows (GraphPad Software, USA). Normality of the data distributions was checked by the Kolmogorov–Smirnov or the Shapiro–Wilk test. The analysis was performed by Unpaired Student's *t*-test for normally distributed data and Mann-Whitney *U* test when data did not display a normal distribution. The effect of acute morphine injection on the mean firing rates of LPGi neurons was analyzed by paired Student's *t*-test (counts were normally distributed). Chi-square test was used to analyze the distribution of LPGi neuronal responses. Data are presented as mean ± standard error of the mean (SEM). In all cases, differences with a $P < 0.05$ were considered statistically significant.

3. Results

3.1. Heterogenous responses of LPGi neurons to acute morphine administration in adult rats receive chronic saline/morphine during adolescence (saline/morphine-treated group)

The effect of acute morphine injection on LPGi neuronal discharge rate was evaluated in adult rats received saline or morphine during

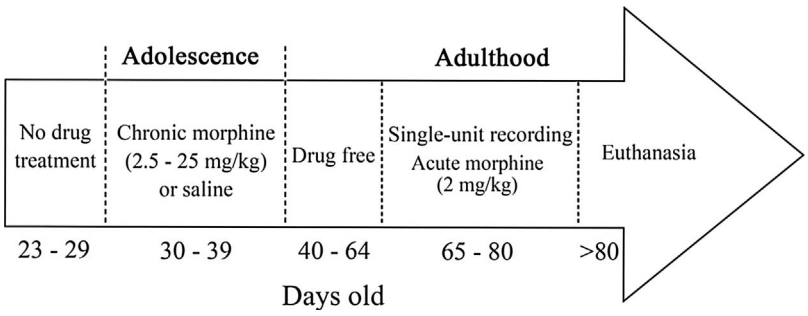

Fig. 1. Schematic representation of the whole experimental procedures. Rats were randomly divided into two experimental groups. Morphine injections coincided with the onset of early adolescence in rats (30 d) and were administered twice a day for 10 consecutive days (30–39 d). All rats were then experienced a drug-free period during which no injections were given (40–64 d). Electrophysiological recording was performed in rats within the range of 65–80 days old, corresponding to the period of adulthood in rats.