Available online at www.sciencedirect.com
ScienceDirect
Neurochemistry International 50 (2007) 386–394
NEUROCHEMISTRY International
www.elsevier.com/locate/neuint

Metabolic changes in rat prefrontal cortex and hippocampus induced by chronic morphine treatment studied *ex vivo* by high resolution ^{1}H NMR spectroscopy

Hongchang Gao [a,b], Yun Xiang [a,b,d], Ninglei Sun [b,c], Hang Zhu [a,b], Yaqiang Wang [a,b], Maili Liu [a], Yuanye Ma [c,*], Hao Lei [a,*]

[a] *State Key Laboratory of Magnetic Resonance and Atomic and Molecular Physics, Wuhan Institute of Physics and Mathematics, Chinese Academy of Sciences, Wuhan 430071, PR China*
[b] *Graduate School, Chinese Academy of Sciences, Beijing 100049, PR China*
[c] *Laboratory of Primate Cognitive Neuroscience, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming 650223, PR China*
[d] *Medicine and Life Science School, Jianghan University, Wuhan 430056, PR China*

Received 12 July 2006; received in revised form 12 September 2006; accepted 18 September 2006
Available online 30 October 2006

Abstract

Ex vivo ^{1}H NMR spectroscopy was used to measure changes in the concentrations of cerebral metabolites in the prefrontal cortex (PFC) and hippocampus of rats subjected to repeated morphine treatment known to cause tolerance/dependence. The results show that repeated morphine exposure induces significant changes in the concentrations of a number of cerebral metabolites, and such changes are region specific. After 10 days of repeated morphine treatment, the concentration of γ-aminobutyric acid (GABA) increased significantly in the PFC ($20 \pm 11\%$), but decreased in the hippocampus ($-31 \pm 12\%$), compared to control. In contrast, the glutamate (Glu) concentrations in both the PFC ($-15 \pm 8\%$) and hippocampus ($-13 \pm 4\%$) decreased significantly. Significant changes were also observed in the concentrations of hippocampal glutamine (Gln), *myo*-inositol, taurine, and *N*-acetyl aspartate. These morphine-induced changes were reversed during a subsequent 5-day withdrawal period. It is suggested that the observed concentration changes for Glu, Gln and GABA are most likely the result of a shift in the steady-state equilibrium of the Gln-Glu-GABA metabolic cycle. Changes in the metabolism of this neurotransmitter system might be part of the adaptive measures taken by the central nervous system in response to repeated morphine exposure and subsequent withdrawal.

Keywords: Morphine; ^{1}H NMR; Rat; Drug dependence; Metabolism; Neurotransmitter

1. Introduction

Repeated exposure to morphine is often accompanied by the development of tolerance and dependence, the exact mechanisms underlying which are not fully understood, but are generally thought to be related to drug-induced neuroadaptation. In recent years, a growing body of evidence has shown that, in addition to the well-studied dopaminergic system, altered glutamatergic and γ-aminobutyric acid (GABA)ergic neurotransmission may also play essential roles in the development of morphine tolerance and dependence. For example, microdialysis studies have shown that acute and chronic morphine treatment alters extracellular concentrations of glutamate (Glu) and GABA significantly in a number of brain regions including nucleus accumbens (NAc) (Sepulveda et al., 2004), prefrontal cortex (PFC) (Hao et al., 2005) and hippocampus (Guo et al., 2005). Morphine treatment causes changes in neuronal Glu release (Nicol et al., 1996), synaptic Glu uptake (Xu et al., 2003), expression of Glu transporters (Mao et al., 2002) and NMDA receptor-mediated neurotransmission (Martin et al., 1999). Morphine treatment has

Abbreviations: PFC, prefrontal cortex; GABA, γ-aminobutyric acid; Glu, glutamate; Gln, glutamine; NAc, nucleus accumbens; GDH, glutamate dehydrogenase; GABA-T, GABA transaminase; PCA, principal component analysis; PC, principal component; Cr, creatine; *m*-Ins, *myo*-inositol; Tau, taurine; NAA, N-acetyl aspartate; Ace, acetate; Asp, aspartate; Lac, lactate; Ala, alanine; MRS, magnetic resonance spectroscopy; VTA, ventral tegmental area

* Corresponding authors. Tel.: +86 2787198542; fax: +86 2787199291.
E-mail addresses: yuanma0716@vip.sina.com (Y. Ma), leihao@wipm.ac.cn (H. Lei).

0197-0186/$ – see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuint.2006.09.012

also been shown to result in decreased GABA release (Capogna et al., 1993; Vaughan et al., 1997), increased GABA uptake, and elevated GABA transporter (i.e., GAT-1) expression in the hippocampus (Hu et al., 2003). Development of morphine tolerance and withdrawal signs after abstinence can be blocked by treatment of NMDA receptor antagonist (Lutfy et al., 1995) and/or GABA receptor agonist (Bexis et al., 2001).

However, there have been few studies of the metabolic consequences of altered glutamatergic and GABAergic neurotransmission occurring during the development of morphine tolerance/dependence. Morphine treatment has been found to induce alterations in cerebral glucose utilization in some brain regions (Beck et al., 1989; London et al., 1990) and in the activities of enzymes such as glutamate dehydrogenase (GDH, EC 1.4.1.2) and GABA transaminase (GABA-T, EC 2.6.1.19) (Kaur and Kaur, 2001) involved in the metabolism of neurotransmitters. Sharma et al. measured cerebral metabolic changes caused by repeated morphine treatment in rat qualitatively using high-resolution nuclear magnetic resonance (NMR) spectroscopy, and observed reduced total (i.e., extracellular + intracellular) Glu concentration in the brain stem and spinal cord of morphine-treated rats (Sharma et al., 2003). Repeated morphine treatment in rat has been shown to result in significantly elevated Glu/GABA concentration ratio in the striatum (Desole et al., 1996).

In the present study, the concentrations of cerebral metabolites in the PFC and hippocampus of rats subjected to repeated morphine treatment and subsequent withdrawal were measured *ex vivo* using high resolution ^{1}H NMR spectroscopy. Two questions are addressed: (1) Will the total concentrations of Glu, GABA and other metabolites change, as a result of altered cerebral metabolism, in response to repeated morphine exposures and subsequent withdrawal? (2) Will the metabolic changes be region-specific? In other words, will the concentration changes observed in the PFC, if any, be different from those found in the hippocampus?

2. Materials and methods

2.1. Animal handling and morphine treatment

Male Sprague–Dawley rats (6–8 weeks old, weighed 200–250 g) were purchased from the Experimental Animal Center, Affiliated Hospital of Kunming Medical College, Kunming, Yunnan, China. The animals were group-housed and maintained on a 12/12 h light–dark cycle with free access to food and water. All procedures were conducted in strict adherence to the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

The rats received intraperitoneal injections of morphine hydrochloride (10 mg/ml, Shenyang First Pharmaceutical Factory, China) at a dose of 10 mg/kg body weight twice per day at 12-h intervals for 1 ($n = 6$), 6 ($n = 6$) or 10 ($n = 24$) consecutive days. Ten days of treatment at this dosage is known to cause significant morphine tolerance and dependence (Yang et al., 2004). A sub-set ($n = 18$) of the rats subjected to 10 days of morphine injection went on to a withdrawal period, for which morphine administration was ceased on the 11th day and abstinence lasted for 1, 3 or 5 days (withdrawal groups, $n = 6$ for each group). The rats in the control group received daily injections of the same amount of physiological saline for 10 consecutive days ($n = 6$).

2.2. Preparation of brain extracts

The rats were sacrificed by decapitation 12 h after their last morphine injection or on the given day during the withdrawal period (for the withdrawal groups). Specimens of bilateral PFC and whole hippocampus were dissected immediately, snap-frozen in liquid nitrogen and stored at −80 °C until use. The frozen tissue was weighed and ground in a mortar cooled in liquid nitrogen using a pestle. Ice-cold perchloric acid solution (12% (g/ml), ∼1.0 ml for the PFC and ∼0.5 ml for the hippocampus, depending on the amount of the sample) was added into the mortar drop by drop, and the frozen mixture was ground again. The pulverized mixture was then transferred to a Dounce homogenizer and homogenized at 4 °C after thawing. The homogenate was centrifuged at 10,000 × *g* for 20 min at 4 °C. The supernatant was extracted, neutralized with 1.0 M KOH, centrifuged again to eliminate perchlorate salts, and lyophilized for about 36 h. The metabolite mixture obtained was then weighed and dissolved in 0.6 ml of 99.5% D_2O for NMR spectroscopy.

2.3. NMR experiments

All ^{1}H NMR experiments were carried out on a Varian INOVA-500 spectrometer at 25 °C using a 90° flip angle, with a spectral width of 8000 Hz and 32 K data points. The acquisition time was 2 s per scan, and an additional 15 s relaxation delay (including 3 s water signal presaturation) was used to ensure full relaxation. The number of scans was 256. The spectra were zero-filled to 64 K, corrected manually for phase and baseline and referenced to the chemical shift of the lactate methyl peaks at 1.33 ppm. Peak area integration was performed using standard routines provided by the XWINNMR software package (Version 2.1, Bruker).

2.4. Data analysis

Using trimethylsilyl-propionic-2,2,3,3d_4-acid (TSP) as the external reference, the concentrations of metabolites were determined from the spectra and normalized to the weight of the freeze-dried metabolite mixture. The average concentration of each metabolite in the control group was calculated and taken as the reference (i.e., 100%) against which to measure the changes in metabolite concentrations in other groups. Absolute metabolite concentrations in the unit of mmol/kg wet tissue weight were also calculated for the control rats and the results were listed in Table 1 and compared with data reported in the literature.

Principal component analysis (PCA) was employed to see whether or not the spectra obtained from different groups could be classified according to their spectral characteristics (Lindon et al., 2001). For PCA, the sub-spectrum (−0.01 to 4.51 ppm) of each sample was automatically data-reduced to 113 integral segments of equal length (0.04 ppm each) using an AMIX software package (Version 2.1, Bruker). The individual integrals in each sub-spectrum were normalized to the sum of all integrals before being entered into the software

Table 1
Concentrations (mean ± S.D.) of cerebral metabolites (mmol/kg wet tissue weight) in the PFC and hippocampus of the control rats measured by *ex vivo* ^{1}H NMR spectroscopy

Metabolite	Present study			Literature data[a]
	Integrated region (ppm)	PFC	Hippocampus	Forebrain
Glu	2.38–2.32	8.47 ± 0.39	7.93 ± 0.31	8.61 ± 0.15
Gln	2.47–2.42	3.27 ± 0.27	2.83 ± 0.17	2.44 ± 0.11
GABA	2.31–2.28	1.96 ± 0.11	2.18 ± 0.13	1.02 ± 0.14
m-Ins	3.64–3.59	4.92 ± 0.19	5.71 ± 0.29	4.42 ± 0.05
Tau	3.44–3.40	5.69 ± 0.32	5.42 ± 0.53	5.05 ± 0.10
NAA	2.02–2.00	8.28 ± 0.46	8.04 ± 0.45	8.62 ± 0.13
Cr	3.02–3.03	7.96 ± 0.43	8.06 ± 0.50	7.98 ± 0.09
Glu/GABA	–	4.34 ± 0.36	3.64 ± 0.20	–
Gln/Glu	–	0.39 ± 0.04	0.36 ± 0.03	–

Literature results measured by *in vivo* ^{1}H MRS are also listed for comparison.
[a] Pfeuffer et al. (1999).