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Original Article

Morphine and sufentanil treatment alters microRNA profiling in neurons

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Abstract: Opioids are morphine-like substances that bind to opioid receptors and are often used to relieve pain. Whether this kind of drugs, including natural product morphine and synthetic product sufentanil, have a protective or destructive effect on nervous system is still unclear. Here, we investigated the alterations of microRNA expression profile after morphine and sufentanil treatment in cultured mouse neurons using microarray. Five microRNA upregulated and 15 microRNA downregulated after morphine treatment and 5 microRNA upregulated and 5 microRNA downregulated after sufentanil treatment with the cutoff of 2-fold change. Our data suggest that microRNA alteration may be involved in opioid drugs' effects in the central nervous system.

Keywords: Morphine, sufentanil, microRNA, anesthetics, Alzheimer's disease, profiling

Introduction

Morphine ($C_{17}H_{19}O_3N$) is widely used as a treatment for diarrhea and pain. Besides pain-relieving, morphine was also reported to play other roles in many physiological processes [1]. It has been controversial whether morphine is protective or destructive to neurons in the central nervous system. It has been reported that chronic exposure to morphine can induce autophagy in both neuroblastoma cell lines and animal brains [2]. It has been reported that morphine has protective effects. For instance, morphine significantly reduces LPS- or 1-methyl-4-phenylpyridinium-induced dopaminergic neurotoxicity [3]. Besides its role in the central nervous system, morphine is also reported to have anticancer activity and immune response suppressing activity [4, 5]. However, the exact mechanism of morphine activity is still under investigation. Sufentanil is a synthetic opioid drug 500 times as potent as morphine. The main clinical use of sufentanil is to relieve acute pain [6]. Sufentanil can also be used for a sedation [7]. MicroRNAs are small non-coding RNA molecules that modulate RNA silencing and post-transcriptional of gene expression [8]. Currently over one thousand miRNAs are found in human genome [9]. Although functions of most microRNAs are still unclear, these molecules involved in almost all physiologic processes, including anesthesia.

In the present study, the alterations of microRNA profile after morphine and sufentanil treatment in primarily cultured mouse hippocampal neurons were revealed. We found that 5 microRNAs were upregulated and 15 microRNA downregulated after morphine treatment. Five microRNAs were upregulated and 5 microRNA downregulated after sufentanil treatment with the cutoff of 2-fold change.

Materials and methods

Cell cultrue and treatments

Tissue collection was done according to Peking University Animal Care and Use Committee as previously described [10]. We dissected hippocampus from new-born C57B6J mice with 0.25% trypsin (Solarbio, T1300) at 37°C. The trypsin was inactivated by Dulbecco's Modified

Table 1. MicroRNAs upregulated in morphine treatment

ProbeSetID	Morphine	Control	P/C ratio	Transcript ID (Array Design)
20500392	8.094431	2.909423	2.7821	mmu-miR-204-3p
20504222	83.58266	28.24932	2.9587	mmu-miR-7b-3p
20510753	22.96201	7.361329	3.1193	mmu-miR-433-5p
20525945	6.475597	2.909423	2.2257	mmu-miR-378d
20536095	4.881892	2.34391	2.0828	mmu-miR-6958-3p

Table 2. MicroRNAs downregulated in morphine treatment

ProbeSetID	Propofol	Control	P/C ratio	Transcript ID (Array Design)
20500261	611.916	1335.383	0.4582	mmu-miR-125a-5p
20500273	45.50671	118.5827	0.3838	mmu-miR-9-5p
20500367	5.659225	14.19647	0.3986	mmu-miR-183-5p
20500377	5.659225	12.251	0.4619	mmu-miR-188-5p
20500408	1.212106	3.354533	0.3613	mmu-miR-203-3p
20500431	2.917209	7.361329	0.3963	mmu-miR-30e-3p
20500658	1.709454	8.943122	0.1911	mmu-let-7d-3p
20500891	1.00621	2.025005	0.4969	mmu-miR-21a-5p
20500964	15.21411	31.11573	0.489	mmu-miR-331-3p
20500988	0.8072776	1.64992	0.4893	mmu-miR-340-5p
20501140	2.256797	5.109654	0.4417	mmu-miR-138-1-3p
20504162	1.285014	3.936171	0.3265	mmu-miR-486-3p
20515477	3.829435	10.26738	0.373	mmu-miR-3102-3p
20515485	1.403396	2.909423	0.4824	mmu-miR-3107-3p
20526117	0.8072776	1.64992	0.4893	mmu-miR-7075-5p

Eagle Medium with 10% fetal bovine serum (Gibco, 10099141) after 12 minutes and then dissociated mechanical trituration with 5 pipette. The cells were plated at 10 per well on 6-well plate precoated with poly-l-lysine. The plates was incubated with 5% CO_2 at 37°C. Neurons were cultured in Neurobasal medium with 1% B27, 1% fetal calf serum, 500 mM Glutamax and 1x antibiotic Pen-Strep (Invitrogen). Medium was half replaced every 3 days. Neurons were treated with 3 μM morphine and 7.77 nM sufentanil especially at 8 DIV for 1 h followed by 1 h recovery. Then the total RNAs of neurons were collected for microRNA profiling.

RNA preparation

Neuronswerelysed by 1 ml Trizol reagent (Ambion, 135301) per well. Mixture were separated into 3 phases by centrifuging at 12,000 g for 15 minutes at 4°C. The aqueous phase were transferred to fresh tube and 400 μl 2-propanol was added. The mixture was centrifugated at 12,000 g for 10 minutes at 4°C after 10 minutes standing to get RNA precipitate. RNA precipitate was washed by 1 ml 75% ethanol and centrifuged at 12,000 g for 5 minutes at 4°C. The RNA was dried by air and then dissolved by Rnase-free water.

MicroRNA profiling

MicroRNA expression levels measurement were conducted by CapitalBio (Beijing, China) microarray service by using genechip Affymetrix miRNA 4.0. The data analysis was done by CapitalBio.

Statistical evaluation

Statistical significance was assessed by one-way analysis of variances (ANOVA). The t test was applied for statistical significance as the post-hoc analysis following ANOVA. A p value of less than 0.05 was used as an indicative of statistical significance.

Results

Microarray of miRNAs was performed on pooled hippocampal neuronal samples (n=3 for control, n=3 for morphineand n=3 for sufentanil treatment group) using FlashTagHSR procedure. According to our data, 20 microRNA expression levels altered after morphine treatment compared with control group. Among this 20 microRNA, 5 were upregulated (**Table 1**) and 15 were downregulated (**Table 2**) with the cut-off of 2-fold change. Among all the 3164 examined microRNA, mmu-miR-195a-3p, mmu-miR-494-3p and mmu-miR-1949 were most dramatically upregulated, and mmu-miR-331-3p, mmu-miR-3102-3p and mmu-let-7d-3p were most dramatically downregulated (**Figure 1**). After sufentanil treatment, 5 of the microRNA examined were upregulated (**Table 3**), which are mmu-miR-204-3p, mmu-miR-7b-3p, mmu-miR-433-5p, mmu-miR-378d, mmu-miR-6958-3p. Five of the microRNA tested were downregulated (**Table 4**), which are mmu-miR-9-5p, mmu-miR-6239, mmu-miR-6923-5p, mmu-miR-7075-5p and mmu-miR-7653-5p (**Figure**

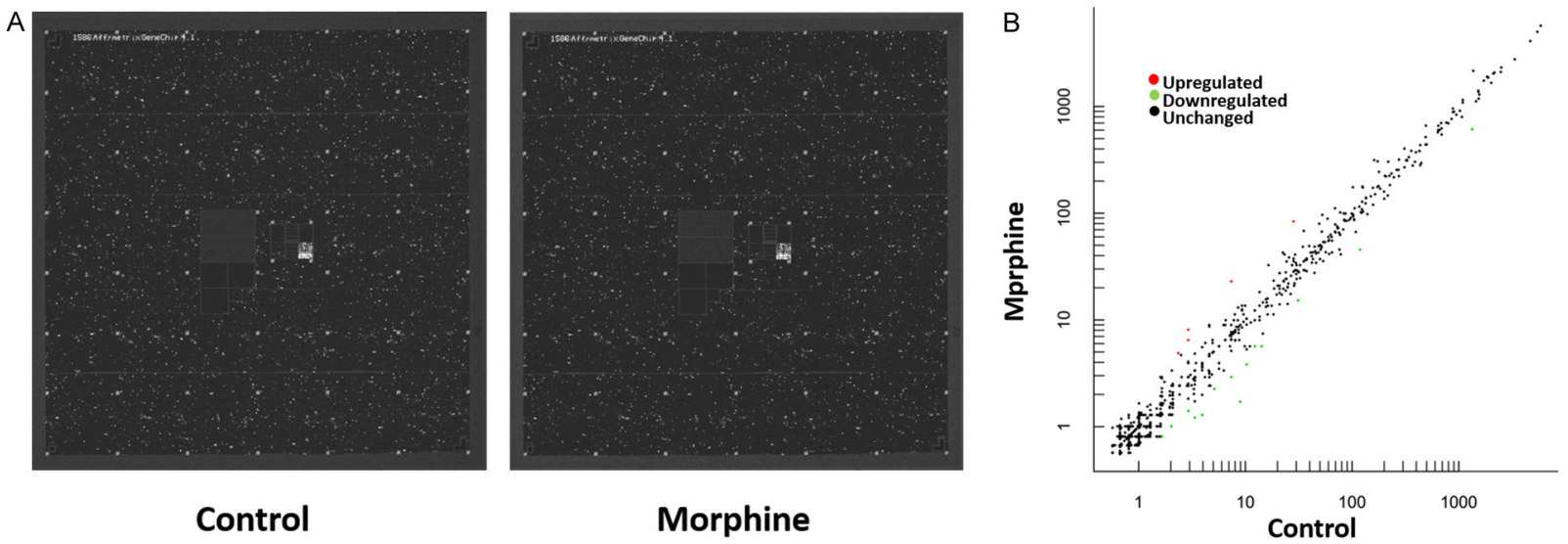

Figure 1. Alterations of microRNAs after morphine treatment. A. Raw data images of control group and morphine treated group. B. Scatter plot of upregulated (red dots), downregulated (green dots) miRNAs and unchanged microRNAs (black dots) with morphine treatment compared with control group.

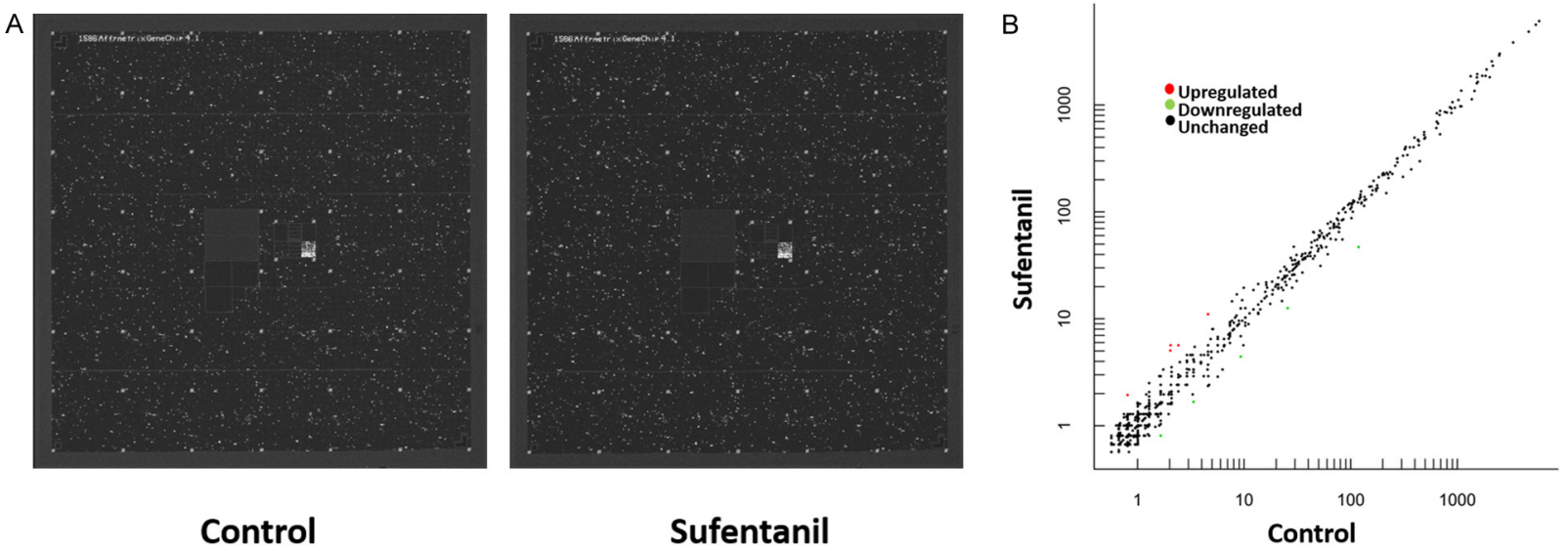

Figure 2. Alterations of microRNAs after sufentanil treatment. A. Raw data images of control group and sufentanil treated group. B. Scatter plot of upregulated (red dots), downregulated (green dots) miRNAs and unchanged microRNAs (black dots) with sufentanil treatment compared with control group.

Table 3. MicroRNAs upregulated in sufentanil treatment

ProbeSetID	Morphine	Control	P/C ratio	Transcript ID (Array Design)
20500410	1.936199	0.8072776	2.3984	mmu-miR-204-3p
20501152	11.04598	4.590311	2.4064	mmu-miR-7b-3p
20502245	5.051769	2.030707	2.4877	mmu-miR-433-5p
20524709	5.659225	2.045775	2.7663	mmu-miR-378d
20525878	5.659225	2.415209	2.3432	mmu-miR-6958-3p

Table 4. MicroRNAs downregulated in sufentanil treatment

ProbeSetID	Morphine	Control	P/C ratio	Transcript ID (Array Design)
20500273	46.91599	118.5827	0.3956	mmu-miR-9-5p
20524328	4.431632	9.295676	0.4767	mmu-miR-6239
20525807	12.58434	25.63422	0.4909	mmu-miR-6923-5p
20526117	0.8072776	1.64992	0.4893	mmu-miR-7075-5p
20528524	1.672354	3.354533	0.4985	mmu-miR-7653-5p

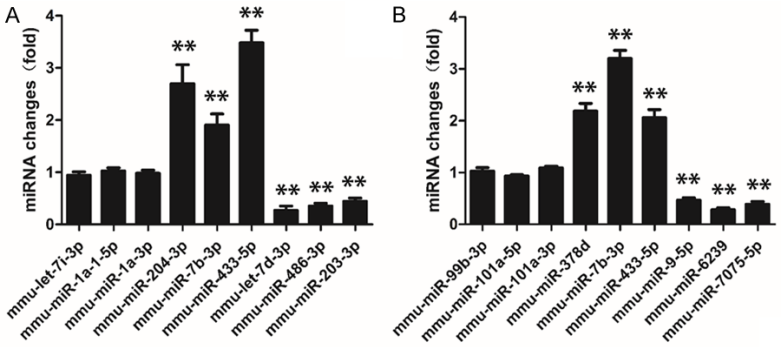

Figure 3. Confirmation of altered miRNAs detected by microarray analysis. A. Most dramatically upregulated and downregulated miRNAs with morphine treatment in microarray assay were confirmed by RT-PCR. Mmu-let-7i-3p, mmu-miR-1a-1-5p and mmu-miR-1a-3p were used as unchanged controls. B. Most dramatically upregulated and downregulated miRNAs with sufentanil treatment in microarray assay were confirmed by RT-PCR. Mmu-miR-99b-3p, mmu-miR-101a-5p, mmu-miR-101a-3p were used as unchanged controls. In both A and B, data represented mean ± SEM (n=3). **: $p<0.01$ compared with the control.

2). The alterations identified in microarray were confirmed by quantitative RT-PCR for both morphine- and sufentanil-treatments (**Figure 3**).

Discussion

Opioids are often used to relieve pain, and could be addictable [11]. Morphine is the most famous drug among opioids. Morphine was first isolated between 1803 and 1805 from the plant opium poppy and has been studied for years. Morphine induces analgesia through activation of opioid receptor in the central nervous system [12]. Although morphine is known by its antinociceptive property, it involved in non-opioid receptor pathways as well. High concentration morphine treatment may lead to hyperalgesia and allodynia by regulating nociceptive substance P (SP) and glutamate concentration [13]. It has been widely reported that morphine induces apoptosis of several kinds of cells [14]. Although a number of pathways which morphine-like opioid participate in were revealed by previous researches,the regulation of microRNAs has not been looked into. In the present study, we find that 5 microRNA were upregulated and 15 microRNA downregulated after morphine treatment. Five microRNA were upregulated and 5 microRNA downregulated after sufentanil treatment with the cut-off of 2-fold change. Our data shed a light on how opioid may play roles via regulating microRNAs.

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Disclosure of conflict of interest

None.

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