Effects of Chronic Morphine Treatment on Metabolism of Glutamate and GABA in Rat Prefrontal Cortex Studied by Ex Vivo High Resolution ^{1}H NMR Spectroscopy

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Introduction Morphine is an opioid of abuse that can elicit many psychoactive effects including euphoric effects, decreased anxiety, altered alertness and intoxication [1]. The mechanisms underlying morphine addiction are poorly understood, but thought to be related to adaptive alterations of the brain in response to repeated morphine exposures [2]. Prefrontal cortex (PFC), a brain region with high densities of opioid receptors, is involved in the reinforcing effects of drugs of abuse [3]. Previous studies conducted in human subjects and animals have consistently shown that chronic opioid exposure reduces neuronal activities in the PFC [4]. In this study, tissue extracts obtained from the brain of morphine-treated rats were studied by ^{1}H nuclear magnetic resonance (NMR) spectroscopy to investigate the effects of chronic morphine treatment and subsequent withdrawal on cerebral metabolism in the PFC, particularly, that of the glutamate (Glu)-GABA neurotransmitter systems.

Materials and Methods Male SD rats (200-250 g, N=6 for each group) were injected with morphine hydrochloride (10 mg/ml, 10 mg/kg body weight) twice one day with a 12 hour interval for 1, 6 and 10 days, respectively. For the rats in the withdrawal groups, morphine administration was ceased on the 11th day and the withdrawal periods lasted for 1, 3 and 5 days, respectively. The control rats received daily injection of the same amount of saline for 10 consecutive days. The rats were decapitated at 12hrs after their last morphine injection or on the given day after withdrawal. Specimens of the PFC were collected, frozen in liquid nitrogen and grinded in a cold mortar. Cerebral metabolites were extracted using a 12% perchloride acid solution. The tissue extracts were neutralized by KOH, lyophilized and dissolved in 99.5% D_2O for high resolution ^{1}H NMR spectroscopy, which was carried out on a Varian INOVA 500 spectrometer with a relaxation delay of 12 s and 256 transients. Spectral processing and integration for all peaks were performed using standard routines provided by the Bruker XWINNMR software. Statistical analysis was carried out using a Dunnett's two-sided test for multiple comparisons.

Results Typical ^{1}H NMR spectra of the PFC extracts are shown in Fig. 1. Chronic morphine treatment caused a progressive increase in GABA level, and concurrent decreases in glutamate level and Glu/GABA ratio in the PFC (Fig. 2). All changes were statistically significant on day 10 of morphine treatment and gradually recovered towards the control levels after withdrawal.

Discussion Dynamic changes of Glu and GABA levels observed during the course of chronic morphine treatment and the subsequent withdrawal period may be the results of neuroplastic alterations associated with the adaptive responses of the Glu-GABA neurotransmitter systems to morphine exposure and detoxification, respectively. Activation of opioid receptors evoke GABAergic activities in the PFC [5], which provide inhibitory influences on the glutamatergic pyramidal neurons and result in decreases of their activities [6]. In addition, GABAergic and glutamatergic activities in the PFC are affected indirectly by dopaminergic projections from the ventral tegmental area (VTA). It is known that activation of opioid receptors in the VTA increases GABAergic activities and decreases glutamatergic activities in the PFC [7, 8]. Long-term alterations in GABAergic and gluatmatergic activities in the PFC induced by repeated morphine exposures could have been the underlying causes for the observed changes in GABA and Glu levels (Fig. 2). The observation that the Glu/GABA ratio is reduced in the PFC of morphine-dependent rats is also consistent with the previous results showing that the neuronal and metabolic activities in the PFC of drug addicts are attenuated when compared to control [4]. Interestingly morphine-induced changes of Glu and GABA found in this study are exactly the opposite of those discovered in patients with major depression diseases who reportedly show decreased GABA level and elevated Glu level in the occipital cortex [9]. Whether or not such observations represent a sheer coincidence needs further investigation. If not, this could be seen as an evidence supporting a possible link between morphine addiction and major depression in terms of pathological changes of Glu-GABA neurotransmitter systems in these diseases.

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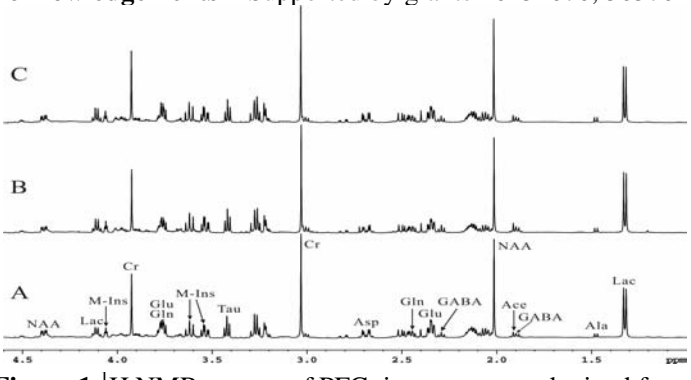

Figure 1 ^{1}H NMR spectra of PFC tissue extracts obtained from a control rat (A) and morphine-treated rats on day 10 of treatment (B) and on 3d of the withdrawal period (C), respectively.

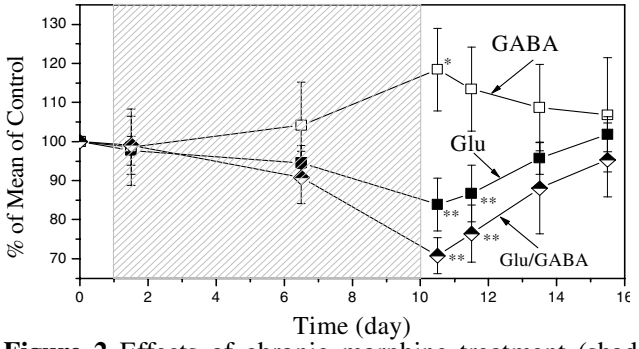

Figure 2 Effects of chronic morphine treatment (shaded area) and subsequent withdrawal (day 11-15) on glutamate (Glu) and GABA levels and Glu/GABA ratio in rat PFC. $^*p<0.05$ and $^{**}p<0.01$ compared to control (day 0).

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