CHAPTER I

Introduction

Rationale of the study

Currently, the abuse of the amphetamine-type stimulants (ATS), amphetamine, methamphetamine, and ecstasy, has been increased over the last decade and spreading throughout the world (United Nations Office on Drugs and Crime, 2004; 2007). Over the 2000 to 2005 period, it was reported that the use of amphetamine and methamphetamine forms reached to 64 percent of ATS use, and half of ATS use took place in Southeast Asia (United Nations Office on Drugs and Crime, 2007). In Thailand, between 1999 and 2002, the number of methamphetamine abusers increased by approximately 290 percent (Office of the Narcotics Control Board, 2002), and the percentage of the population use methamphetamine in 2004 was 0.7 percent of population between the ages 15 and 64 years (United Nations Office on Drugs and Crime, 2006). This trend leads to serious situation in Thailand to make social and economic problems especially the cost of health care (e.g. medication, behavioral treatments, job training and social services). Presently, no effective pharmacotherapy for methamphetamine abuse has demonstrated efficacy for acute- or long- term use.

Methamphetamine, a class of the amphetamine-type stimulants (ATS), is structurally and functionally similar to amphetamine. Although, both stimulants are highly addictive and have similar neurobiochemical effects. In addiction research, the common mechanism of methamphetamine has also been encompassed with amphetamine. Amphetamine is a dopamine agonist that also impact directly on dopaminergic system (Moore, 1997), producing loss of dopaminergic marker such as dopamine content (O'Dell et al., 1991; Fukumura et al., 1998), tyrosine hydroxylase (TH) (Fukumura et al., 1998; Cappon et al., 2000), vesicular monoamine transporter (VMAT) (Guilarte 2003), and dopamine transporter (DAT) (Eisch et al., 1992; McCann et al., 1998; Volkow et al., 2001; McCann and Rucaurte, 2004). These alterations indicate of dopaminergic axons and terminal damage, which confirmed by histological studies (Broening et al., 1997; Fukumura et al., 1998; Bower and

Schmued, 2006). Recently, studies showed that both humans (Simon et al., 2002; Kamei et al., 2005) and animals (Bisagno et al., 2002; Belcher et al., 2006) exposed to neurotoxic methamphetamine are impaired in behavioral tasks which involved are in learning and memory functions. Some studies found a decrease in dopamine transporters in the striatum related to motor and cognitive impairments in methamphetamine abusers (Sekine et al., 2001; Volkow et al., 2001; Chang et al., 2002). However, Belcher and colleagues did not find a reduction of dopamine and serotonin transporters after methamphetamine-induced objective recognition impairment (Belcher et al., 2006). These suggest that other factors apart from monoamine terminal injury may contribute to the methamphetamine-induced cognitive impairment. Yet it is not clear whether the neurotoxicity found after methamphetamine exposure is associated with impairments of cognitive functions.

Since, it is known that glutamate play a critical role in cognition such as learning and memory functions (Saal and Malenka, 2005). An interaction between dopamine and glutamate has been documented to be involved in drug dependent pathway (Tzschentke and Schmidt, 2003; Kelley, 2004; Reid and Lingford-Hughes, 2006; Howell and Kimmel, 2007). It has been reported that dysfunction of dopamine system can cause alteration in glutamate transmission after methamphetamine-induced neurotoxicity (Mark, 2004). However, no evidence has been demonstrated whether or not methamphetamine administration affects glutamatergic system. Therefore, it is important to evaluate the effect of methamphetamine on glutamate transmission which regard to possible learning and memory impairments.

Glutamate is released from glutamatergic neurons and acts on various types of glutamate receptors. N-methyl-D-aspartate (NMDA) receptors, a subtype of glutamate receptors, have been substantially implicated in mediating neuronal plasticity especially learning and memory (Castellano et al., 2001; Riedel et al., 2003; Perez-Otano and Ehlers, 2005). They are present mainly on postsynaptic neurons throughout the brain (Tovar and Westbrook, 1999), and functional NMDA receptor requires at least one of NMDAR1 subunits together with other subunits (Dingledine et al., 1999; Nishi et al., 2001). Thus, it is assumed that NMDAR1 is an indicator for NMDA receptor function. Preclinical and preliminary clinical observations suggest that NMDA receptors are involved in drug addiction mechanism (Eisch et al., 1996;

Yamamoto et al., 1999; Miyamoto et al., 2004), and NMDA receptor antagonists are also potential candidates to treat withdrawal syndromes from opioid (Bisaga et al., 2001; Krystal et al., 2003b), sedative (Krystal et al., 2003b) and alcohol (Krystal et al., 2003a; 2003b). Recent studies demonstrated that NMDAR2B antagonist attenuate withdrawal-induced toxicity in alcohol pre-treated neuronal cultures (Nagy et al., 2004). Although, less is known about methamphetamine dependence on NMDA receptor and pharmacotherapeutic approaches for treatment of methamphetamine dependence, a study has shown a loss of NMDA receptors following withdrawal from methamphetamine (Eisch et al., 1996). Moreover, Yamamoto et al (1999) demonstrated a reduction in NMDAR1 expression in the striatum of methamphetamine-sensitized rats. Altogether, glutamate has been consistently found to be one of primary neurochemical substrates involved in drug dependence. Thus, it is very interesting to study mechanisms of glutamate transmission especially NMDAR1 receptor after methamphetamine administration.

Methamphetamine produces increases in extracellular glutamate concentrations in several regions of brain (Abegava et al., 1994; Nash and Yamamoto, 1992; Stephan and Yamamoto, 1994; Staphan and Yamamoto, 1995; Rocher and Gardier). Accumulating of glutamate in the synaptic cleft may induce excitotoxicity neuronal damage via overstimulation of glutamate receptors (Longuemare and Swanson, 1995). Keeping extracellular glutamate below excitotoxic levels by glutamate transporters is believed to be the major regulatory and neuroprotective mechanism. Dysfunction of glutamate transporters may induce cellular dysfunction and cell death (Struzynska et al., 2005). A selective loss of neuronal glutamate EAAT3 transporter and glial glutamate EAAT2 transporter has been observed in the pathogenesis of ischemic brain and epilepsy (Mathern et al., 1999) which also reflects an increase of extracellular glutamate. Interestingly, recent study has reported that a non-selective excitatory amino acid transporter (EAAT) inhibitor, L-trans-pyrrolidine-2,4-dicarboxylic acid (PDC), and a specific EAAT2 inhibitor, (+/-)-threo-3-methylglutamic acid (MG), attenuated methamphetamine toxicity (Hayase et al., 2003). Therefore, it has been suggested that not only EAAT2 but also the other EAATs may contribute to the methamphetamine-induced toxicity. However, no evidence has been shown whether methamphetamine administration influences the

expression of neuronal glutamate transporter (EAAT3) in brain regions which are involved in learning and memory.

There are evidences that several brain regions in corticolimbic-striatal network including hippocampal formation, frontal cortex and striatum are thought to be crucial in the circuitry of drug dependence (Kelly, 2004). All of these regions possess high distribution of both glutamate NMDAR1 receptors and neuronal glutamate transporter (EAAT3) which are markers for glutamatergic transmission. Although effects of methamphetamine on glutamatergic transmission have been proposed as a one of mechanism of methamphetamine neurotoxicity, in particularly, glutamate NMDAR1 receptor and neuronal glutamate transporter (EAAT3) in methamphetamine toxicity conditions has yet to be investigated. It is of interest to assess the alterations in glutamate NMDAR1 receptor and neuronal glutamate transporter (EAAT3) in rat brain after methamphetamine administration. Thus, the objective of the study was to investigate the influence of methamphetamine on the expression of the glutamate NMDAR1 receptor and neuronal glutamate transporter (EAAT3) in rat hippocampal formation, frontal cortex and striatum.

Purpose of the study

A general experiment objective

This experiment is designed to evaluate expression of the glutamate receptor (NMDAR1) and neuronal glutamate transporter (EAAT3) in methamphetamine dependence.

Specific objectives

1. To determine the alteration of the glutamate receptor (NMDAR1) in the hippocampal formation, frontal cortex and striatum after acute and chronic methamphetamine administration in rats.

2. To determine the alteration of the neuronal glutamate transporter (EAAT3) in the hippocampal formation, frontal cortex and striatum after acute and chronic methamphetamine administration in rats.

3. To determine the possible mechanisms underlying the alteration of the glutamate receptor (NMDAR1) and the neuronal glutamate transporter (EAAT3) in the hippocampal formation, frontal cortex and striatum after acute and chronic methamphetamine administration in rats.

Significance of the study

1. To provide the information about glutamatergic transmission in hippocampal formation, frontal cortex and striatum in methamphetamine dependence.

2. To provide the understanding about the possible mechanisms underlying the glutamatergic transmission in methamphetamine dependence.

Scope of this study

1. All animals used in this experiment were only adult male rat weighted approximately 200-250 grams.

2. All injections should be done at the same period to avoid influence of circadian rhythm.

3. All stresses should be avoided to prevent the influence of stress.

4. In all experiments, control and experimental groups should be performed in parallel at the same period to avoid the effect of seasonal changes.

Hypotheses

1. If methamphetamine exerts an alteration of glutamatergic transmission in hippocampal formation, frontal cortex and striatum, the results in animal treated-group should be shown either higher or lower expression of glutamate receptor (NMDAR1) in hippocampal formation, frontal cortex and striatum than control group.

2. If methamphetamine exerts an alteration of glutamatergic transmission in hippocampal formation, frontal cortex and striatum, the results in animal treated-group should be shown either higher or lower expression of neuronal glutamate transporter (EAAT3) in hippocampal formation, frontal cortex and striatum frontal cortex than control group.

3. If the alteration of glutamate receptor (NMDAR1) and neuronal glutamate transporter (EAAT3) has been found in hippocampal formation, frontal cortex and striatum after methamphetamine administration, the mechanism of glutamate transmission may be involved in methamphetamine dependence.