THE EFFECT OF METHAMPHETAMINE ON SEIZURE THRESHOLD AND EPILEPTOGENESIS

by

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ABSTRACT

Epilepsy, one of the most common chronic neurological disorders, is characterized by recurrent spontaneous seizures. Seizures result from many causes including genetic or developmental defects, traumatic brain injury, substance abuse, infection, fever, metabolic disturbances, withdrawal symptoms and space-occupying lesions in the brain.

Memamphetamine (METH), a schedule II drug, leads to seizures that are mostly due to overdose. In recent years, there has been a rapid increase in METH-related emergencies, in which seizures are one of the most common and fatal symptoms. It is hypothesized in this dissertation that METH affects seizure threshold and epileptogenesis through its modulation of monoamine systems. This hypothesis was tested by completing the following specific aims.

Specific Aim 1 investigated the effect of METH on seizure threshold in two acute electroconvulsant (tonic hindlimb extension & 6-Hz psychomotor) and two acute chemoconvulsant (pentylentetrazol & kainic acid) models. The results suggest that METH alters seizure threshold in a model-specific manner: METH increased the tonic hindlimb extension threshold while it decreased the threshold for 6-Hz psychomotor seizures and pentylentetrazol-induced clonus; the threshold for kainic acid-induced seizures was not affected by METH treatment.

Specific Aim 2 explored the role of dopaminergic receptor modulation in METH-induced change of electroconvulsant seizure threshold. The data demonstrated that the D-1 antagonist SCH-23390 displayed a synergistic effect with METH in modulating tonic hindlimb extension and 6-Hz psychomotor seizure threshold,
whereas an antagonistic effect was observed between the D-2 antagonist eticlopride and METH. Moreover, eticlopride blocked the SCH-23390/METH synergism in a dose-dependent manner.

Specific Aim 3 assessed the effect of METH on amygdala kindling acquisition and brain monoamine levels. Multiple METH treatment facilitated epileptogenesis by enhancing the amygdala kindling acquisition rate. Serotonin (5-HT) was found to be depleted in the limbic system (amygdala and hippocampus) and striatum. Dopamine (DA) concentration was reduced in the striatum. However, there was no change in norepinephrine (NE) levels.

In summary, METH modulates seizure threshold and facilitates epileptogenesis, which depends on its interaction with monoaminergic neurotransmission. Results obtained from this study provide important information to our understanding of the mechanisms underlying METH-related convulsions.
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CHAPTER 1

INTRODUCTION

Methamphetamine (METH), a schedule II drug, causes seizures in both humans (Lan et al., 1998) and animals (Hanson et al., 1999). According to the Drug Abuse Warning Network (DAWN, U.S. Department of Health and Human Services), METH-related emergency room visits per 100,000 population have dramatically increased in recent years: from 4 in 1999 to 36.7 in 2005 (Fig. 1.1). In those emergencies, convulsions are common and often fatal (Lan et al., 1998; see NIDA, 2002). A single seizure could progress to status epilepticus, which is often resistant to standard anticonvulsant drugs (Lowenstein et al., 1993). Thus, the studies of METH's effect on seizures in animal models are clinically relevant. It is hypothesized in this dissertation that METH modulates animal seizure threshold and epileptogenesis through its impact on monoamine systems.

Epilepsy, Seizures and Epileptogenesis

Epilepsy is one of the most common neurological disorders characterized by spontaneous recurrent seizures. It affects approximately 2.3 million people in the United States (Hawkins et al., 2005) and more than 50 million people worldwide (World Health Organization, 2005). Seizures, excessive synchronized excitation of neuronal networks, are classified into two types, i.e., partial and generalized. Partial (focal) seizures are confined to a discrete part of the brain (focus) and subdivided into simple partial (consciousness is retained) and complex partial seizures (consciousness
Figure 1.1. METH-related emergency department visits per 100,000 population from 1999 to 2005 in U.S.A. Source: Drug Abuse Warning Network, U.S. Department of Health and Human Services.
is impaired or lost). Some partial seizures may spread to other brain areas and become secondarily generalized. Generalized seizures are those that involve both hemispheres from the outset. They produce loss of consciousness, either briefly or for a longer period of time, and are subcategorized into several major types: absence, myoclonic, clonic, tonic, tonic-clonic, and atonic seizures.

Known causes of seizures include genetic or developmental defects (for reviews, see Berkovic et al., 2006; Guerrini and Marini, 2006), traumatic brain injury (see Gupta and Gupta, 2006), CNS infections (see Vezzani and Granata, 2005), fever (see Mewasingh, 2006), metabolic disturbances (Anuradha et al., 2004), substance abuse (see Brust, 2006), drug toxicity (Swiader et al., 2003; Chemg and Wong, 2005), withdrawal from CNS depressants (see Hillbom et al., 2003; Rathlev et al., 2006) and space-occupying lesions in the brain such as brain tumors (Yeates et al., 2003; Atig et al., 2006).

Epileptogenesis is defined as a sequence of events that converts a normal neuronal network into a hyperexcitable network which produces recurrent spontaneous seizures (see Lowenstein, 1996; Dudek and Clark, 2004; see Dichter, 2006). There are multiple mechanisms contributing to epileptogenesis (see Najm et al., 2001). Genetic (see Lerche et al., 2005) or developmental (Sanchez and Jensen, 2001) deficits can lead to idiopathic epilepsy. Acquired epilepsy results from altered neurotransmission: increased activation of glutamatergic system (Jin et al., 2006) and/or decreased GABA-mediated inhibition (Sloviter, 1991). In addition, neuronal loss (see DeLorenzo and Sun, 2006), structural reorganization (Sutula et al., 1989; Represa et al., 1993), nonsynaptic (Naus et al., 1991) and other mechanisms (for reviews, see Lowenstein, 1996; Acharya, 2002) contribute to epileptogenesis.
Animal Seizure and Epileptogenesis Models

In 1972, Purpura and his colleagues published "Experimental Models of Epilepsy - A Manual for the Laboratory Worker" (Raven Press, New York), which summarized the available models for epilepsy research at that time. Since then, tremendously more in vivo and in vitro epilepsy/seizure models have been proposed and established (see Pitkanen et al., 2006). The studies utilizing these models have significantly advanced our understanding of epilepsy/seizure mechanisms and facilitated anticonvulsant drug development (see White, 1997).

The currently available animal seizure models include electroconvulsant, chemoconvulsant, audiogenic and genetic-caused seizures. The present study assessed the effect of METH on seizure threshold in two electroconvulsant (tonic hindlimb extension and 6-Hz psychomotor) and two chemoconvulsant (pentylenetetrazol and kainic acid) models (Table 1.1.), and epileptogenesis was evaluated in the well-documented amygdala kindling model of partial seizures secondarily generalized (Goddard et al., 1969; McIntyre et al., 2002).

The tonic hindlimb extension can be induced by a strong and generalized electrical stimulation of the brain (Krall et al., 1978). Experimental techniques commonly used in rodents deliver a high-frequency (60 Hz), short-duration (0.2 s) current through transcorneal or transaural electrodes. Intense currents stimulate large portions of the brain and induce tonic flexion followed by tonic hindlimb extension and clonus accompanied by a loss of posture (Krall et al., 1978, Mares and Hubova, 2006). In 1939, Merritt and Putnam utilized a variant of this model, maximal electroshock (MES), to successfully establish the anticonvulsant activity of phenytoin (Merritt and Putnam, 1939). Since then, the MES has become a critical test for screening anticonvulsant drugs effective against generalized tonic-clonic (grand mal)
Table 1.1. The four seizure threshold testing models applied in this dissertation. The electroconvulsant model parameters (current frequency, duration and intensity), chemoconvulsant doses, seizure phenotypes and outcome measures for each model are summarized.

<table>
<thead>
<tr>
<th>Seizure models</th>
<th>Currents or drugs</th>
<th>Seizure phenotypes</th>
<th>Outcome measure(s)</th>
</tr>
</thead>
</table>
| **Tonic hindlimb extension** | Frequency: 60 Hz  
Duration: 0.2 s  
Varying intensities | Hindlimb extended 180 degrees at the plane of the body  
Varying intensities | CC50 (intensity of current which induces tonic hindlimb extension in 50% of the rats tested) |
| **6-Hz psychomotor**  | Frequency: 6 Hz  
Duration: 3 s  
Varying intensities | Twitching of the vibrissae, jaw and forelimb clonus, dorsal neck flexion, elevated tail, rearing and falling | CC50 (intensity of current which induces partial limbic seizure in 50% of the rats tested) |
| **Pentylenetetrazol** | 35 mg/kg, s.c.    | Hypoactivity, partial clonus (face, head, forelimb), generalized clonus              | Incidence, latency and duration of clonic seizures                                  |
| **Kainic acid**       | 20 mg/kg, i.p.    | Immobility, wet dog shakes, facial clonus, salivation, forelimb clonus and, occasionally, tonic activity | Incidence, latency and duration of clonic seizures                                  |
seizures (Krall et al., 1978; White et al., 1995a & b), which are thought to inhibit seizure spread through brainstem structures (Woodbury and Esplin, 1959; Browning et al., 1981 & 1985; Browning, 1985 & 1987; Gale and Browning, 1988; Applegate et al., 1991).

The 6-Hz psychomotor seizure (6-Hz) model is an alternative low frequency (6 Hz), long duration (3 s) stimulation paradigm resulting in partial limbic seizures. The phenotypes of 6-Hz psychomotor seizures include jaw and forelimb clonus, immobility, twitching of the vibrissae and a Straub-tail (Barton et al., 2003). The 6-Hz model has been applied to identify compounds with unique anticonvulsant profiles (Barton et al., 2001 & 2003). For example, the anticonvulsant drug levetiracetam has been proven efficacious in the 6-Hz model but not other acute electroconvulsant models (Barton et al., 2001 & 2003). Likewise, several glutamate receptor modulators [e.g., the mGlu2/3 (LY379268 and LY389795) and Group III (L-AP4) metabotropic receptor agonists] demonstrate anticonvulsant activity in the 6-Hz rather than MES model (Barton et al., 2003). In contrast, phenytoin is inactive in the 6-Hz test but is active in the MES model (Barton et al., 2001).

Pentylenetetrazol (PTZ), a GABA receptor antagonist, has been extensively utilized in animals to induce generalized clonic or tonic-clonic seizures (Orlof et al., 1949; Swinyard et al., 1989; White et al., 1995a). Low doses of PTZ cause clonic seizures that originate from forebrain structures (Loscher and Ebert, 1996), and high doses result in generalized tonic-clonic seizures by activating networks in the brainstem (Browning and Nelson, 1986). In addition, repeated low doses of PTZ may induce status epilepticus (SE) in immature rats (Pereira de Vasconcelos et al., 1995; Nehlig and de Vasconcelos, 1996). PTZ-induced clonus represents a routine test for
anticonvulsant drug screening (Swinyard et al., 1989; White et al., 1995a). In this dissertation, PTZ was administered at the subconvulsive dose of 35 mg/kg (s.c.) and did not induce clonus in control rats.

Kainic acid (KA), a common neurotoxin (McGeer et al., 1978), is used to induce partial limbic seizures and status epilepticus in rodents (Sperk, 1994; Leite et al., 2002; Velisek, 2006). The KA-induced seizures are characterized by immobility, wet dog shakes, facial clonus (repetitive chewing, blinking), salivation, forelimb clonus and, occasionally, tonic activity (Silveira et al., 2002). KA-induced seizure excites the limbic regions including the hippocampus and amygdala (Silveira et al., 2002), causes neuronal loss and synaptic reorganization particularly in the hippocampus (Ben-Ari, 1985; Cavazos et al., 2004), and, therefore, serves as a valuable model of partial limbic seizures with secondary generalization (Ben-Ari, 1985).

Kindling, a phenomenon first discovered by Graham Goddard in 1967 (Goddard, 1967), is described as the progressive intensification of behavioral and electrographic seizures as a result of repeated, low-intensity electrical stimulations to particular brain structures (Goddard et al., 1969). The progression begins initially with minimal or no change in behavior or brain electrical activity as measured by electroencephalograph (EEG) recordings. As subsequent stimulations are delivered, long and high-frequency afterdischarges (ADs) associated with convulsive responses evolve. Once an animal has reached a stable kindled state, the hyperexcitability of the network seems to be permanent and is associated with a decreased afterdischarge threshold (ADT), i.e., the minimal current intensity required for eliciting an AD (Racine, 1972a).

As a complement to traditional seizure models, kindling provides a good model for complex partial seizures with secondary generalization and epileptogenesis of temporal lobe epilepsy (TLE) (see Bertram, 2007). Since its discovery, numerous
researchers have utilized the kindling model to study the mechanisms underlying epileptogenesis. In addition, kindling is a useful model for studying neural plasticity and memory (Hannesson and Corcoran, 2000).

Kindling can be induced by simulating multiple forebrain structures in the brain including the amygdala, hippocampus, piriform and entorhinal cortices (Goddard et al., 1969; Racine et al., 1972; Racine, 1972b & 1975). The amygdala is the most commonly used site for kindling because of relatively fewer (1-15) stimulations required to reach fully kindled status (Goddard et al., 1969). Usually, the basolateral amygdala of rats is electrically stimulated once per day. Seizure severity is assessed using the Racine scale, i.e., "0", no seizure; "1", mild head nodding with or without jaw chomping; "2", 5-10 seconds of unilateral forelimb clonus; "3", forelimb clonus; "4", rearing with forelimb clonus; "5", rearing and falling with forelimb clonus (Racine, 1972b). As an animal becomes kindled, the seizure severity enhances as estimated by the seizure score and the afterdischarge duration (ADDs, Fig. 1.2) increases.

**Methamphetamine and Monoamine Neurotransmission**

Methamphetamine (METH), also known as "ice", "chalk", "crystal" and "speed", is a member of the amphetamine group of sympathomimetic amines. METH has a strong stimulating effect on the CNS and is highly addictive. As a schedule II drug, METH is under strict regulatory control and has limited medical use. It is sometimes prescribed for ADHD (Attention Deficit Hyperactivity Disorder) in children and narcolepsy with the brand name Desoxyn (Mitler, 1994; Halpern, 1999).

METH can be taken orally, intranasally (snorting the powder), intravenously, or by smoking. Smoking or intravenous injection of METH produces a brief and intense sensation. A long-lasting high is reported by those who orally ingest or snort METH.
Figure 1.2. The electroencephalograph (EEG) of amygdala kindled seizure. Electroencephalograph signal was recorded through the electrode implanted in rat basolateral amygdala during kindling and analyzed by the software Manager MP 100 (version 3.5.3, Biopac Systems, Inc.). The EEG trace includes baseline activity, evoked afterdischarge and recovering phase. Afterdischarge duration (ADD) is the period from the onset to the end of electrographic seizure. Longer ADDs reflect more severe seizures.
In addition to euphoria and the rush that it produces, METH causes a broad range of bodily changes such as enhanced attention and activity, increased respiration, heart rate and blood pressure, decreased appetite and fatigue, depression, hyperthermia and convulsions (see NIDA, 2002).

METH has long been known to be a modulator of monoamine neurotransmission. Monoamines are neurotransmitters that contain one amino group connected to an aromatic ring by a two-carbon chain (-CH2-CH2-). All monoamines are derived from aromatic amino acids like phenylalanine, tyrosine, histidine, tryptophan, and the thyroid hormones by the action of aromatic amino acid decarboxylase enzymes (Schwartz, 2000). The monoamines are a large family that includes the catecholamines [i.e., dopamine (DA), norepinephrine (NE) and epinephrine (Epi)], serotonin (5-HT), melatonin, histamine, thyronamines and trace amines (Schwartz, 2000). The synthesis and metabolism of DA, 5-HT and NE are diagramed in Fig. 13 (see Cooper et al., 1996). The dopamine transporter (DAT), serotonin transporter (SERT), and norepinephrine transporter (NET) are localized to the outer cell membrane and the vesicular monoamine transporters 1 and 2 (VMAT-1 and VMAT-2) are found in the vesicular membrane (see Cooper et al., 1996). These transporters mediate monoamine reuptake and vesicle loading, respectively. The activation of monoamine receptors results in a wide range of physiological changes and is implicated in certain pathological and psychopathological conditions (Kilian and Frey, 1973, Mayert et al., 1975; Glennon, 1990; see Cooper et al., 1996; see Dunlop and Nemeroff, 2007).

Dopaminergic receptors are a class of G-protein coupled receptors that include D-1 like (D-1 and D-5 receptors) and D-2 like (D-2, D-3 and D-4 receptors) families (see Dziedzicka-Wasylewska, 2004; Werkman et al., 2006). The selective D-1 and
Figure 1.3. The synthesis and metabolism of dopamine (DA), serotonin (5-HT) and norepinephrine (NE). AADC: aromatic amino acid decarboxylase; COMT: catechol-O-methyltransferase; DA: dopamine; DBH: dopamine-P-hydroxylase; DHPG: dihydroxyphenylethleneglycol; DOPAC: dihydroxyphenylacetic acid; 5-HIAA: 5-hydroxyindoleacetic acid; 5-HT: 5-hydroxytryptamine or serotonin; 5-HTP: 5-hydroxytryptophan; HVA: homovanillic acid; MAO: monoamine oxidase; MHPG: 3-methoxy-4-hydroxyphenylethleneglycol; 3-MT: 3-methoxytyramine; NE: norepinephrine; TH: tyrosine hydroxylase; TPH: tryptophan hydroxylase.
D-2 receptor antagonists employed in this dissertation are SCH-23390 (Iorio et al., 1983; Sandoval et al, 2002) and eticlopride (Hall et al., 1985; Prinssen et al., 2004), respectively. Seven distinct families of 5-HT receptors have been identified (5-HT1 - 5-HT7) and subpopulations have been described for several of those (Glennon, 1990 & 2003). Norepinephrine (NE) exerts its activity through two families of adrenergic receptors: alpha and beta families, both of which are coupled with G-proteins (Strasser et al., 1992).

METH inhibits monoamine oxidase (Robinson, 1985), likely reverses the function of monoamine transporters which has been demonstrated for amphetamine (see Fleckenstein et al, 2000) and redistributes vesicular monoamine transporter-2 (VMAT-2) (Riddle et al, 2002). These effects result in an accumulation of monoamines in the presynaptic terminal and subsequent release into the synaptic cleft. The influence of METH on dopaminergic neurotransmission is illustrated in Fig. 1.4.

A single large dose or multiple smaller doses of METH cause long-term reduction of striatal DA and hippocampal 5-HT (Kogan et al, 1976; Seiden et al, 1976; Ricaurte et al, 1980; Marek et al, 1990; Fukumura et al, 1998). This monoaminergic neurotoxicity induced by METH is contributed to factors including reactive oxygen and/or nitrogen species, hyperthermia, aberrant monoamine, glutamate transmission and mitochondrial disruption (Albers and Sonsalla, 1995; see Riddle et al, 2006), and the toxicity is age-dependent, i.e., it is less in neonatal and adolescent rats compared to adult rats (Lucot et al, 1982; Truong et al, 2005; Rau et al, 2006). Moreover, METH-induced neurotoxicity includes nerve terminal degeneration and neuronal apoptosis (Cadet et al, 1997 & 2003; see Riddle et al, 2006).
Figure 1.4. Diagram illustrating the influence of METH on dopaminergic neurotransmission. METH inhibits monoamine oxidase (MAO), reverses the function of dopamine transporters (DAT) and redistributes vesicular monoamine transporters. These lead to elevated DA level in the presynaptic terminal and its subsequent release into the synaptic cleft. Dopamine in the synaptic cleft binds to D-1 and D-2 receptors, which are coupled with stimulatory and inhibitory G-proteins, respectively. The compounds SCH-23390 and eticlopride are selective D-1 and D-2 antagonists, respectively. In addition, there are also presynaptic D-1 receptors mediating negative feedback to the dopaminergic neurons.
The Roles of DA, 5-HT and NE in Seizures

A large body of evidence supports an important role of monoamines in seizure threshold modulation (Kilian and Frey, 1973; Maynert et al., 1975; Lints and Nyquist-Battie, 1985; Chauvel and Trottier, 1986; Lerner-Natoli, 1987). Early clinical observations showed that DA agonists were beneficial in some forms of reflex epilepsy (Quesney et al., 1980). Moreover, the anticonvulsant effect of the dopaminergic agonist apomorphine was observed in genetically seizure-prone DBA/2 mice (Anlezark and Meldrum, 1975) and on photically induced seizures in baboons (Meldrum et al., 1975). However, conflicting experimental results were reported on the effects of DA agonists and antagonists in electroshock, kindling and chemoconvulsant seizures models (Callaghan and Schwark, 1979; Kleinrokh et al., 1978).

The discovery of selective D-1 and D-2 agonists/antagonists provided a new means for addressing the role of DA in seizure modulation. Numerous studies have supported the anticonvulsant action of D-2 agonists against amygdala kindled seizures in rats, electroconvulsions in mice and reflex seizures in genetic models (air blast-induced seizures in gerbils) (Loscher and Czuczwar, 1986). Further investigation suggests that the anatomical and receptor specificity of DA action in basal ganglia is mediating its effect on seizures. Specifically, the D-1 receptor activation in the substantia nigra pars reticulata (SNr) decreases pilocarpine-induced generalized seizure threshold, while the D-2 activation in the striatum leads to anticonvulsant effect (Turski et al., 1990).

Serotonin (5-HT) has demonstrated consistent anticonvulsant activity in various seizure models (Lerner-Natoli, 1987). Depletion of 5-HT with p-chlorophenylalanine (PCPA) reduces PTZ-induced seizure threshold (Alexander and Kopeloff, 1970),
while high tryptophan diets prevent PTZ-induced seizures in animals (Cabral-Filho et al., 1987). Amygdala kindling is blocked by the electrical stimulation of median raphe (5-HT origin site) (Siegel and Murphy, 1979), whereas the destruction of serotonergic terminals in amygdala with neurotoxins results in the facilitation of amygdala kindling (Lerner-Natoli, 1987). In addition, the duration of epileptiform afterdischarge is prolonged by 5-HT antagonists methysergide and cyproheptadine, and reduced by 5-HT precursor 5-hydroxytryptophan (Krip and Vazquez, 1971). These data suggest that 5-HT inhibits seizure generation, while a decrease in 5-HT function increases seizure susceptibility (see Peterson and Alberson, 1982; Burley and Ferrendelli, 1984; Waterhouse, 1986).

Norepinephrine (NE) is also engaged in the generation, development or maintenance of seizure activity in animals (see Corcoran, 1981). Numerous experimental manipulations that compromise central noradrenergic function result in a proconvulsant effect (see McIntyre, 1981). For example, the selective reduction of NE level by DSP-4 [N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine] enhances the rate of hippocampal kindling in laboratory rats (Bortolotto and Cavalheiro, 1986), thereby suggesting an inhibitory effect of NE on kindling acquisition.

**Hypothesis and Dissertation Overview**

It is hypothesized in this dissertation that METH modulates seizure threshold and epileptogenesis in animals by manipulating monoamine neurotransmission.

Results described in Chapter 2 demonstrate that METH affects seizure threshold in a model-specific manner. For example, METH enhanced the tonic hindlimb extension threshold, while it decreased the threshold for 6-Hz psychomotor and pentylenetetrazol (PTZ)-induced seizures. However, the threshold for kainic acid (KA)-induced seizures was not affected by METH.
The studies outlined in Chapter 3 test the hypothesis that dopaminergic receptor modulation is involved in the METH-induced change of electroconvulsant seizure threshold. The blockade of D-1 and D-2 receptors results in synergistic and antagonistic effects with METH on modulating electroconvulsant threshold, respectively. Moreover, the D-2 antagonist eticlopride blocked the synergy between SCH-23390 (D-1 antagonist) and METH in a dose-dependent manner.

Results described in Chapter 4 indicate that METH enhances epileptogenesis by facilitating amygdala kindling acquisition. Furthermore, serotonin (5-HT) was depleted in the limbic system (amygdala and hippocampus) and striatum. Dopamine (DA) was reduced in the striatum, while norepinephrine (NE) levels were unaltered. By blocking METH-induced monoamine depletion, we attenuated the facilitating effect of METH on amygdala kindling acquisition.

Understanding the effect of METH on seizure threshold and epileptogenesis is useful for discovering the mechanisms and better treatments for METH-related convulsions and their sequelae.
References


CHAPTER 2

METHAMPHETAMINE AFFECTS RAT SEIZURE THRESHOLD
IN A MODEL-SPECIFIC MANNER

Introduction

Methamphetamine (METH) is a highly addictive (schedule II) drug which is derived from amphetamine and has strong effect in the CNS (see Fleckenstein et al., 2007). METH use has surged over the last decade in North America (Barr et al., 2006), which causes increasing health and social problems (Sommers et al., 2006). According to the statistics of Drug Abuse Warning Network (DAWN, U.S. Department of Health and Human Services), the number of METH-related emergency room visits per 100,000 population has increased more than 9-fold in recent years: from 4 in 1999 to 36.7 in 2005. In those emergency cases, seizures were reported as serious clinical manifestations which could be fatal (Lan et al., 1998; see NIDA, 2002). In Lan’s study, all patients who died from acute METH intoxication developed seizures, whereas only 23% of those who survived underwent seizures (Lan et al., 1998). A recent epidemiologic study involving 106 young adult METH users indicates that seizure is one of the most serious METH-related health problems (Sommers et al., 2006).

METH causes clonic seizures in mice following intracerebroventricular (i.c.v.) administration (Hanson et al., 1999). However, acute METH reduces the incidence of electroconvulsant tonic seizures in mice (Nakamura et al., 1993). Repeated METH treatment even enhances this acute METH-induced inhibition of tonic extension
(Nakamura et al., 1993). Moreover, prenatal METH exposure increases the flurothyl-induced seizure susceptibility (Slamberova, 2005), while it has no effect on kainic acid (KA)-induced seizures (Slamberova and Rokyta, 2005). Therefore, METH seems not to have unifying effect on seizure susceptibility.

The present study systemically assessed the effect of METH on rat seizure threshold in two electroconvulsant (tonic hindlimb extension and 6-Hz psychomotor and two chemoconvulsant [pentylenetetrazol (PTZ) and kainic acid (KA)] models.

**Methods**

**Subjects**

Male Sprague-Dawley rats purchased from Charles River Laboratories (Wilmington, MA) were used for all experiments in this study. Rats were housed in Comparative Medicine Center facilities (University of Utah) maintained at 22°C and a 12-h light/dark cycle, with food and water provided ad libitum. All experiments were conducted between 9am and 5pm to exclude the influence of diurnal and circadian rhythms on animal seizure threshold (see Quigg, 2000). All protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Utah, and are in accordance with the National Institutes of Health *Guidelines for the Care and Use of Laboratory Animals*.

**Drugs**

(±)Methamphetamine hydrochloride was provided by the National Institute on Drug Abuse (Rockville, MD) and doses were calculated as the free base. Pentylenetetrazol (PTZ) and kainic acid (KA) were purchased from Sigma (ST. Louis, MO). All solutions were made in 0.9% saline and vortexed for 3 min before injection. Drug solutions were freshly prepared on the day of each experiment. Ketamine
hydrochloride (100 mg/kg, i.p.; Abbott Laboratories, N. Chicago, IL) and xylazine (10 mg/kg, i.p.; Sigma, St. Louis, MO) were used for rat anesthesia before surgery.

Electroconvulsant seizure threshold testing

Electroconvulsant seizures were induced by delivering stimulation current to rats (80-100 g) through transcorneal electrodes. A drop of tetracaine (0.5%) was administered to the eyes of each rat just before testing. Two different stimulation protocols were used in an effort to differentiate the effects of METH on tonic hindlimb extension and 6-Hz psychomotor seizures. Tonic hindlimb extension was induced by a stimulator delivering current of high frequency (60 Hz) and short duration (0.2 sec) (Woodbury and Davenport, 1952); current of strong intensity caused a tonic-clonic flexion-extension sequence that starts with tonic forelimb extension, followed by hindlimb flexion and terminates in full tonic hindlimb extension (180 degrees to the torso) (Toman, 1951; Peterson, 1998; White et al., 2002). The 6-Hz psychomotor seizure was elicited by a Grass S48 stimulator delivering currents of lower frequency (6 Hz) and longer duration (3 sec). A 6-Hz psychomotor seizure is characterized by rhythmic face movements, forelimb clonus, dorsal neck flexion, rearing and falling, elevated tail (Straub tail) and/or transient gait wobbliness/ataxia (Barton et al., 2001).

The seizure threshold was determined according to the "staircase" procedure (Swinyard, 1972) wherein the individual animal's response to a given stimulus intensity determines the subsequent current intensity to be tested for the next animal in the group. Based on the data, a convulsive current (CC) curve of the group was generated; the CC50, i.e., convulsive current intensity that induces seizures in 50% of the animals and represents seizure threshold, was estimated by Probit analysis.
Assessment of chemoconvulsant seizures

PTZ (35 mg/kg, s.c.) or KA (20 mg/kg, i.p.) was administered to rats (80-100 grams) 1 h after METH (15 mg/kg, s.c.)/saline injection. Individual rats were placed into isolation cages after PTZ injection and observed over the course of the next 30 min for the presence or absence of clonus (White, 1998). Fisher's exact test and Student's t-test were applied to compare the clonus incidence and latency, respectively, between saline- and METH-treated groups.

Rats treated with KA were placed in isolation cages and observed for the next 3 h for behavioral seizures. Seizures were scored based on Racine scale; i.e., "0", no seizure; "1", mild head nodding with or without jaw chomping; "2", 5-10 sec of unilateral forelimb clonus; "3", forelimb clonus; "4", rearing with forelimb clonus; "5", rearing and falling with forelimb clonus (Racine, 1972). The comparison of incidence and latency of stage 4/5 seizures between control and METH-treated groups were conducted with Fisher's exact test and Student's t-test, respectively.

Rats weighing 300-320 g were used to study the long-term effect of METH. Animals were administered four METH doses at 2-h intervals (4x10 mg/kg, s.c). An equivalent amount of saline was administered to rats in the control group. Three days later, sterile surgeries were conducted under ketamine/xylazine anesthesia to place recording electrodes on the anterior, medial and posterior surfaces of rat brain (Kriz et al., 2003). The electrode headpieces were anchored to the rat skull with screws and dental acrylic cement. After one week of recovery, individual rats were placed in isolated cages and their implanted electrodes were connected to Grass model SD9 amplifier (Astro-Med; Grass Instrument Division, West Warwick, RI) for electroencephalograph (EEG) recording. The behaviors and EEG of rats were
continuously monitored for the next 30 min or 3 h after PTZ (35 mg/kg, s.c.) or KA (20 mg/kg, i.p.) treatment, respectively. The seizure incidence was compared between saline- and METH-treated rats with Fisher’s exact test, and the seizure latency and duration with Student’s t-test.

Statistical analyses

Probit analysis was applied to estimate and compare the CC50 values between control (saline-treated) and experimental (METH-treated) groups. Fisher’s exact test was used to for comparison of seizure incidence. Student's t-test was employed to assess the seizure latency and duration difference. Values are expressed as mean ± S.E.M. (standard error of the mean), and significance was defined as \( p < 0.05 \).

Results

The effect of METH on tonic hindlimb extension seizure threshold

Multiple METH doses (4 x 10 mg/kg, s.c.) markedly enhanced the tonic hindlimb extension threshold 1 h posttreatment in rats weighing 80-100 g. The CC50 of METH-treated group, i.e., convulsive current intensity inducing tonic hindlimb extension in 50% of METH-treated rats, was found to be 88.3 ± 4.3 mA, which is significantly higher than that in saline-treated group: 37.4 ± 1.8 mA (\( p < 0.01 \), Fig. 2.1 A). No difference in the CC50 was observed 24 h posttreatment: 35.9 ± 2.3 mA and 31.4 ± 2.3 mA in saline- and METH-treated groups, respectively (\( p > 0.05 \), Fig. 2.1B).

The effect of METH on 6-Hz psychomotor seizure threshold

METH treatment (4 x 10 mg/kg, s.c.) decreased the 6-Hz psychomotor seizure threshold 1 h posttreatment in rats weighing 80-100 g, i.e., 76.1 ± 1.9 mA vs. 57.8 ± 1.2 mA in saline- vs. METH-treated group, respectively (\( p < 0.01 \), Fig. 2.2A).
**Figure 2.1.** The effect of METH on tonic hindlimb extension seizure threshold. Electrical stimulation (60 Hz, 0.2 sec, varying current intensities) was delivered to rats via transcorneal electrodes to determine the presence or absence of tonic hindlimb extension. The CC50 (convulsive current intensity which induces tonic hindlimb extension in 50% of the rats tested) was estimated by Probit analysis. (A) Multiple METH treatment (4x10 mg/kg, s.c.) increased CC50 for tonic hindlimb extension 1 h posttreatment (means ± S.E.M.; *p < 0.01; n = 34, 36; saline-, METH-treated groups). (B) Multiple METH treatment had no effect on tonic hindlimb extension threshold 24 h posttreatment (p > 0.05; n = 15, 11; saline-, METH-treated groups).
A

Tonic Hindlimb Extension
(1 h posttreatment)

CC50 (mA)

saline
METH

* 37.4 ± 1.8 mA
* 88.3 ± 4.3 mA

B

Tonic Hindlimb Extension
(24 h posttreatment)

CC50 (mA)

saline
METH

* 35.9 ± 2.3 mA
* 31.4 ± 2.4 mA
This effect was still present 24 h posttreatment: 81.7 ± 2.8 mA vs. 69.0 ± 1.8 mA in saline- vs. METH-treated groups \((p < 0.01, \text{Fig. 2.2B}).\)

The effect of METH on PTZ-induced clonic seizures

The chemoconvulsant PTZ at the low dose of 35 mg/kg (s.c.) did not cause clonus in saline-treated rats weighing 80-100 g. However, it induced clonic seizures in 6 out of 8 (75%) rats 1 h after METH (15 mg/kg, s.c.) treatment. Moreover, 2 out of the 6 seized rats displayed multiple clonic seizures (Table 2.1 A). These results suggest that acute METH decreases PTZ-induced clonic seizure threshold.

The long-term effect of multiple METH doses on PTZ-induced seizures was assessed in adult rats weighing 300-320 g. Ten days after multiple METH treatments (4 x 10 mg/kg, s.c), the incidence of clonus to an acute PTZ challenge (35 mg/kg, s.c.) was 3 out of 6 (50%) and the latency was 14.7 ± 4.2 min (mean ± S.E.M.). These results in METH-treated rats were similar to those in saline-treated animals [incidence: 4 out of 6 (66.7%); latency: 17.9 ± 7.0 min] (Table 2.1B). Furthermore, METH seemed to have no long-term effect on the duration of electrographic seizure associated with PTZ-induced clonus (Fig. 2.3).

The effect of METH on KA-induced seizures

Rats weighing 80-100 g were administered KA (15 mg/kg, i.p.) 1 h after METH or saline treatment. Five out of 6 rats in the METH-treated group developed stage 4/5 seizures. This was similar to the incidence observed in saline-treated rats, i.e., 4 out of 6 \((p > 0.05, \text{Table 2.2A}).\) Likewise, no significant difference was found between controls and METH-treated rats in the latency to the first stage 4/5 seizure (Table 2.2A).

Furthermore, multiple METH treatment (4 x 10 mg/kg, s.c) did not have any
Figure 2.2. The effect of METH on 6-Hz psychomotor seizure threshold. Electrical stimulations (6 Hz, 3 sec, varying current intensities) was delivered to rats via transcorneal electrodes to induce a limbic partial seizure. The CC50 was estimated by Probit analysis (means ± S.E.M.). (A) Multiple METH treatment (4x10 mg/kg, s.c.) decreased the 6-Hz seizure threshold 1 h posttreatment (*p < 0.01; n = 15, 25; saline-, METH-treated groups). (B) The effect of multiple METH treatment on the 6-Hz seizure threshold was still evident 24 h posttreatment (*p < 0.01; n = 15, 29; saline-, METH-treated groups).
A

Partial Psychomotor Seizure (1 h posttreatment)

![Graph showing CC50 (mA) for saline and METH with 76.1 ± 1.9 mA and 57.8 ± 1.2 mA respectively.]

B

Partial Psychomotor Seizure (24 h posttreatment)

![Graph showing CC50 (mA) for saline and METH with 81.7 ± 2.8 mA and 69.0 ± 1.8 mA respectively.]

* Indicates significance.
Table 2.1. The effect of METH on pentylenetetrazol (PTZ)-induced clonic seizures. (A) Single METH treatment (15 mg/kg, s.c.) decreased the threshold for PTZ (35 mg/kg, s.c.)-induced clonus 1 h posttreatment \( p < 0.01, n = 8 \) in each group). (B) Multiple METH treatment (4 x 10 mg/kg, s.c.) had no effect on the incidence, latency, EEG duration of PTZ-induced clonus 10 d posttreatment \( p > 0.05, n = 6 \) in each group). The difference in seizure incidence between saline- and METH-treated groups was analyzed with Fisher’s exact test; Student’s t-test was used for the comparison of the seizure latency and duration.
A. The acute effect (1 h) of single METH treatment (15 mg/kg, s.c.) on PTZ (35 mg/kg, s.c.)-induced seizures

<table>
<thead>
<tr>
<th>Outcome measures</th>
<th>Saline-treated group</th>
<th>METH-treated group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incidence of clonic seizures</td>
<td>0/8 (0 %)</td>
<td>6/8 (75.0 %)</td>
</tr>
<tr>
<td>Latency to onset of the first clonic seizure (mean ± S.E.M.)</td>
<td>N/A</td>
<td>8.3 ± 3.3 min</td>
</tr>
<tr>
<td>Multiple clonic seizures</td>
<td>N/A</td>
<td>observed in 2 rats</td>
</tr>
</tbody>
</table>

B. The long-term effect (10 d) of multiple METH treatment (4 x 10 mg/kg, s.c.) on PTZ (35 mg/kg, s.c.)-induced seizures

<table>
<thead>
<tr>
<th>Outcome measures</th>
<th>Saline-treated group</th>
<th>METH-treated group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incidence of clonic seizures</td>
<td>4/6 (66.7 %)</td>
<td>3/6 (50.0 %)</td>
</tr>
<tr>
<td>Latency to onset of the first clonic seizure (mean ± S.E.M.)</td>
<td>17.9 ±7.0 min</td>
<td>14.7 ± 4.2 min</td>
</tr>
<tr>
<td>Duration of the electrographic seizure (mean ± S.E.M.)</td>
<td>44.7 ±21.5 sec</td>
<td>62.5 ± 28.6 sec</td>
</tr>
</tbody>
</table>
Figure 2.3. PTZ-induced clonic seizures were associated with high frequency electrographic seizure activity. Cortical electrodes were surgically attached to the skull of the rat 3 d after METH (4 x 10 mg/kg, s.c.) or saline treatment. One week later, rats were challenged with an acute dose of PTZ (35 mg/kg, s.c.), and their behaviors and EEG were monitored for the next 30 min. (A) PTZ-induced electrographic seizure in a saline-treated rat. (B) PTZ-induced electrographic seizure in a METH-treated rat. METH-treated rats displayed similar electrographic seizure duration to saline-treated ones (p > 0.05, Student’s t-test).
A. PTZ-induced electrographic seizure in a saline-treated rat

B. PTZ-induced electrographic seizure in a METH-treated rat
long-term effect on KA-induced seizure susceptibility in rats weighing 300-320 g. For example, the incidence of stage 4/5 seizures, latency to the first stage 4/5 seizure and electrographic seizure duration were similar in saline- and METH-treated groups (Table 2.2B; Fig. 2.4).

**Discussion**

The results obtained in the present study indicate that METH alters seizure threshold in a model-specific manner. For example, acute METH treatment elevated the generalized tonic-clonic seizure threshold, while it decreased the 6-Hz psychomotor seizure threshold. In addition, METH acutely decreased PTZ-induced clonus threshold but it did not modify the incidence or duration of KA-induced seizures. Studies by Browning have proposed that there are two sites of seizure origin in the rat, i.e., the forebrain and the brainstem (Browning, 1987). It is thought that convulsions mediated by the forebrain structures originate from the deep prepiriform cortex or the area tempestas (Piredda and Gale, 1985), and their phenotypes include face, forelimb clonus and perhaps rearing and falling (Browning, 1987). Forebrain seizures occur independently of the brainstem, which is based on the observation that intact forebrain electrographic seizures can still be induced after complete forebrain transection (Browning et al., 1993). Forebrain convulsions can be induced by low, systemically administered doses of chemoconvulsants (PTZ and KA) or low electrical simulation (6-Hz stimulation) employed in this study. Thus, the effect of METH on the three models above reflects its influence on forebrain structures: METH-induced decrease of forebrain seizure thresholds suggests that it enhances the forebrain local excitability. Although METH seemed not to affect the incidence or latency of KA-induced seizures, an effect of METH cannot be ruled out because it might be masked by the large variability in the incidence of seizures and latency to onset.
Table 2.2. The effect of METH on kainic acid (KA)-induced seizures. (A) Single METH treatment (15 mg/kg, s.c.) did not affect the incidence or latency of KA-induced stage 4/5 seizures 1 h posttreatment ($p > 0.05$). (B) Multiple METH treatment (4×10 mg/kg, s.c.) had no long-term effect (10 d) on the incidence, latency of KA-induced stage 4/5 seizures, or the latency to the first electrographic seizure ($p > 0.05$). The behavioral seizure was scored based on Racine scale (Racine, 1972). The seizure incidence was compared between saline- and METH-treated groups with Fisher's exact test; Student's t-test was applied to analyze the seizure latency and duration.
A. The acute effect (1 h) of single METH treatment (15 mg/kg, s.c.) on KA (20 mg/kg, i.p.)-induced seizures

<table>
<thead>
<tr>
<th>Outcome measures</th>
<th>Saline-treated group</th>
<th>METH-treated group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incidence of stage 4/5 seizures</td>
<td>4/6 (66.7 %)</td>
<td>5/6 (83.3 %)</td>
</tr>
<tr>
<td>Latency to onset of the first stage 4/5 seizure (mean ± S.E.M.)</td>
<td>49.0 ± 14.4 min</td>
<td>55.2 ± 20.2 min</td>
</tr>
</tbody>
</table>

B. The long-term effect (10 d) of multiple METH treatment (4 x 10 mg/kg, s.c.) on KA (20 mg/kg, i.p.)-induced seizures

<table>
<thead>
<tr>
<th>Outcome measures</th>
<th>Saline-treated group</th>
<th>METH-treated group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incidence of stage 4/5 seizures</td>
<td>5/9 (55.6 %)</td>
<td>4/8 (50.0 %)</td>
</tr>
<tr>
<td>Latency to onset of the first stage 4/5 seizure (mean ± S.E.M.)</td>
<td>53.6 ± 24.0 min</td>
<td>51.8 ± 20.5 min</td>
</tr>
<tr>
<td>Latency to onset of the first electrographic seizure (mean ± S.E.M.)</td>
<td>27.2 ± 25.1 min</td>
<td>34.5 ± 19.8 min</td>
</tr>
</tbody>
</table>
Figure 2.4. KA-induced seizures were associated with high frequency electrographic seizure activity. The behaviors and EEG of rats were monitored for 3 h after acute KA administration (20 mg/kg, i.p.) (A) KA-induced first electrographic seizure in a saline-treated rat. (B) KA-induced first electrographic seizure in a METH (4 x 10 mg/kg, s.c.)-treated rat. There was no significant difference in the latency to the first electrographic seizure between saline- and METH-treated groups ($p > 0.05$, Student's $t$-test).
A. KA-induced first electrographic seizure in a saline-treated rat

B. KA-induced first electrographic seizure in a METH-treated rat
observed in KA-treated rats.

Brainstem structures including pontine reticular formation (Browning, 1987; Gale and Browning, 1988) and nucleus reticularis pontis oralis (Browning et al., 1981 & 1985; Browning, 1985; Applegate et al., 1991) are the proposed site for initiating tonic-clonic convulsions. The most convincing evidence is that rats that receive complete precollicular brain transections at the level above the brain stem still respond to maximal electroshock and display generalized tonic-clonic seizures (Browning and Nelson, 1986). High doses of chemoconvulsants such as PTZ or intense electrical stimulation are thought to directly excite the neuronal substrates in the brainstem that mediate generalized tonic-clonic seizures (Browning and Nelson, 1986; Browning, 1987). Thus, METH's increase of tonic hindlimb extension threshold suggests that the brainstem structures are inhibited by an acute METH challenge. Furthermore, the present study supports Browning's theory that the forebrain and hindbrain seizures can function independently (Browning and Nelson, 1986; Browning et al., 1993) and suggests that they have separate thresholds for seizure initiation. The opposing effects of METH on generalized and local seizures indicate that METH has a differential influence on forebrain and brainstem excitability.

In this study, acute METH lowered the PTZ (35 mg/kg, s.c.)-induced clonic seizure threshold, which is thought to involve forebrain structure activation (Loscher et al., 1996). Interestingly, higher doses of PTZ can produce generalized tonic-clonic seizures (Loscher et al., 1991) which are mediated by the brainstem structures (Browning and Nelson, 1986). These results demonstrate that the forebrain and hindbrain seizure origins can be activated by the same chemoconvulsant at different doses.
Like METH, some antiepileptic drugs display differential pharmacological profiles depending on the seizure model. For example, phenytoin was shown to be anticonvulsant in the maximal electro shock model but inactive in the 6-Hz seizure (Barton et al., 2001). In contrast, levetiracetam and compounds including competitive mGlul (LY367385) receptor antagonist, the mGlu2/3 (LY379268 and LY389795) and Group III (L-AP4) metabotropic receptor agonists were ineffective for tonic hindlimb extension but exerted anticonvulsant activities in the 6-Hz test (Barton et al., 2003). These results are consistent with the idea that forebrain and hindbrain seizures are regulated separately.

In summary, METH has varying effects on seizure threshold depending on the specific animal seizure model. It is postulated to be due to its differential influence on the excitability of forebrain and brainstem structures. It is thought that METH excites the limbic system while it inhibits the brainstem structures such as pontine reticular formation (Browning, 1987; Gale and Browning, 1988) and nucleus reticularis pontis oralis (Browning et al, 1981 & 1985; Browning, 1985; Applegate et al., 1991). Thus, METH facilitates the limbic seizure but prevents the generalized tonic-clonic seizure.

On the neurochemical level, METH alters monoamine transporters and increases extracellular concentrations of dopamine (DA), serotonin (5-HT) and norepinephrine (NE) (see Fleckenstein et al., 2000). Those three monoamines have been reported to modify seizure activity (Briere et al., 1986; Chauvel and Trottier, 1986; Lerner-Natoli, 1987; see Weinshenker and Szot, 2002) and may be involved in METH’s effect on seizure threshold. In order to determine whether the monoamine neurotransmission plays a role in METH-induced seizure threshold change, selective monoaminergic antagonists will be tested for their abilities to modify METH-induced changes of seizure threshold in future research.
References


the mechanism of action of amphetamines. Annu Rev Pharmacol Toxicol
47:681-698.

Gale K, Browning RA (1988) Anatomical and neurochemical substrates of clonic and
tonic seizures. In: Mechanisms of epileptogenesis (Dichter MA, ed), ppl 11-152.
New York: Plenum Press.

Hanson GR, Jensen M, Johnson M, White HS (1999) Distinct features of seizures

channel blockers in kainic acid-induced experimental seizures in rats. Epilepsy

Lan KC, Lin, YF, Yu FC, Lin CS, Chu P (1998) Clinical manifestations and
prognostic features of acute methamphetamine intoxication. J Formos Med
Assoc 97:528-533.

36:139-151.

Loscher W, Honack D, Fassbender CP, Nolting B (1991) The role of technical,
biological and pharmacological factors in the laboratory evaluation of
anticonvulsant drugs. III. Pentylenetetrazol seizure models. Epilepsy Res
8:171-189.

Loscher W, Ebert U (1996) Basic mechanisms of seizure propagation: targets for

Nakamura J, Yamada S, Horikawa Y, Nose I (1993) Changes in the incidence and
duration of electroconvulsions after acute or subchronic treatment with

National Institute on Drug Abuse, U.S. Department of Health and Human Services
02-4210.


Quigg M (2000) Circadian rhythms: interactions with seizures and epilepsy. Epilepsy

Racine RJ (1972) Modification of seizure activity by electrical stimulation. II. Motor

Slamberova R (2005) Flurothyl seizures susceptibility is increased in prenatally
methamphetamine-exposed adult male and female rats. Epilepsy Res


CHAPTER 3

DOPAMINERGIC RECEPTOR MODULATION IS INVOLVED IN THE ACUTE EFFECT OF SINGLE METHAMPHETAMINE TREATMENT ON ELECTROCONVULSANT SEIZURE THRESHOLD

Introduction

Methamphetamine (METH), a schedule II drug, has been reported over several decades to induce or modify seizures (Ellinwood et al., 1973; Nakamura et al., 1993; Hanson et al., 1999). Acute METH intoxication causes convulsions which are severe and even fatal (Lan et al., 1998; see NIDA, 2002). In rodents, METH administration induces behavioral and electrographic seizures (Hanson et al., 1999). Moreover, METH modifies seizures, and the effect varies depending on the METH administration regimen and specific seizure model. Chronic METH treatment reduces the threshold and afterdischarge duration of hippocampal kindled seizures in the cat (Emori et al., 1991), whereas a temporary elevation of seizure threshold followed by its reduction occurs in amygdala kindling (Minabe et al., 1988). Acute METH decreases the incidence and prolongs the duration of tonic hindlimb extension in mice, and these effects are enhanced by repeated METH administration (Nakamura et al., 1993). Furthermore, prenatal METH exposure alters seizure susceptibility in a model-specific manner. For example, prenatal METH exposure decreases the threshold for first fasciculation and clonic seizure caused by flurothyl in male rats (Slamberova, 2005); it decreases the threshold of flurothyl-induced first fasciculation in diestrous rather than proestrous and estrous female rats (Slamberova, 2005). In
contrast, it has no effect on kainic acid (KA)-induced clonic seizures (Slamberova and Rokyta, 2005).

The studies performed here were designed to assess the role of dopaminergic receptor modulation in mediating METH’s effect on electroconvulsant seizure threshold. It has been known that amphetamine analogs, likely including METH, inhibit the membrane-associated dopamine transporter (DAT) (Wayment et al., 1998; see Schenk, 2002) and reverse the direction of DAT (Raiteri et al., 1979; Kahlig et al., 2005; see also Fleckenstein et al., 2000). Moreover, METH reduces vesicular dopamine (DA) uptake from cytoplasm (Brown et al., 2000) and inhibits DA degradation enzymes (Robinson, 1985; see Sulzer et al., 2005). These effects lead to an accumulation of DA in the presynaptic terminal, which is subsequently released into synaptic cleft through DAT (see Sulzer et al., 2005). Dopamine binds to D-1 like and D-2 like receptors (see Kebabian and Calne, 1979) and causes a sequence of pharmacological and toxicological effects (Clark and White, 1987; see Cooper et al., 1996).

Dopamine (DA) has long been known to be a modulator of seizure activity (Kilian and Frey, 1973; see Maynert et al., 1975; Quattrone et al., 1978). Early clinical observations in humans noticed that neuroleptics introduced into psychiatry practice 50 years ago markedly lower the seizure threshold, probably due to dopaminergic hypoactivity (Barsa and Kline, 1955). The comorbidity of schizophrenia and epilepsy observed by epidemiologists is also regarded as a dopamine hypoactivity condition (see Starr, 1996). Early pharmacological studies have reported that the nonselective DA agonist apomorphine (Anden et al., 1967) is beneficial in generalized photosensitive epilepsy (Quesney et al., 1980). Further studies indicate that the administration of L-dopa, the precursor of DA, and the stimulation of DA receptors by
apomorphine exhibit anticonvulsant effects against electroshock, pentylenetetrazol- and audiogenic-induced seizures in mice and rats (see Maynert et al, 1975). Conversely, blockade of DA receptors has been reported to decrease seizure threshold in some animal models (see Maynert et al., 1975; see Sovner and DiMascio, 1978) while it exhibits no effects in other studies (Loscher and Czuczwar, 1986). Therefore, most literature seems to support the anticonvulsant activity of DA neurotransmission. However, there are exceptions depending on the manner by which DA levels are manipulated and the animal seizure threshold model employed. For example, the reduction of striatal dopamine induced by the neurotoxin 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) protected rodents against maximal electroshock-induced convulsions, but not bicuculline- and picrotoxin-induced seizures (Fariello et al., 1987).

The discovery of two dopaminergic receptor families (D-1 and D-2 like) mediating opposing effects on neuronal excitability (see Kebabian and Calne, 1979; Clark and White, 1987; see Starr, 1996) heralded a new era of research on the role of DA in seizures. Experimental work using drugs selective to D-1 and D-2 receptors suggests that the traditional anticonvulsant action of dopamine is attributed to D-2 receptor stimulation in the forebrain (Loscher and Czuczwar, 1986) whereas D-1 receptor activation in the midbrain exhibits proconvulsant effects (Turski et al., 1990; see Starr, 1996).

Based on the literature, it is hypothesized that dopaminergic receptor modulation is involved in the effect of METH on electroconvulsant seizure threshold. This hypothesis was tested using the tonic hindlimb extension and 6-Hz psychomotor seizure models. Selective dopaminergic receptor modulators were tested for their ability to modify METH-induced changes in electroconvulsant seizure threshold. The
results of these studies form the basis of this report.

Methods

Animals

Male Sprague-Dawley rats (80-100 g) were purchased from Charles River Laboratories (Wilmington, MA) and housed under controlled temperature (22°C) and lighting (12-h light/dark cycle). Animals were allowed free access to food and water. All animal experiments were conducted between 9am and 5pm in an effort to minimize the influences of diurnal and circadian rhythms on seizure threshold (see Quigg, 2000). All animal care and experimental protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Utah and are in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*.

Drugs

(+)-Methamphetamine hydrochloride was provided by the National Institute on Drug Abuse (Rockville, MD) and doses were calculated as the free base. The selective dopamine D-1 antagonist, D-2 antagonist and D-2 agonist employed in this study were SCH-23390 (Iorio et al., 1983; Sandoval et al., 2002), eticlopride (Hall et al., 1985; Prinssen et al., 2004) and quinpirole (Kebabian et al., 1997; Truong et al., 2004), respectively. All compounds were purchased from Sigma (St. Louis, MO) and dissolved in 0.9% NaCl solution. Drug solutions were freshly prepared on the day of each experiment. Seizure threshold was determined 1 h after METH/saline treatment. The drugs SCH-23390 (0.5 mg/kg) and eticlopride (0.05, 0.5 or 5 mg/kg) were administered 30 min and 40 min prior to METH/saline injection, respectively. Quinpirole (20 mg/kg) was injected 1 h prior to seizure threshold test.
Seizure threshold test

Electroconvulsant seizure threshold was determined 1 h after METH/saline treatment. Electrical stimulation was delivered to individual rats via silver-coated transcorneal electrodes. Prior to placement of the electrode, a drop of 0.5% tetracaine in 0.9% saline was administered to the eyes of each rat. The tonic hindlimb extension threshold test was conducted with a stimulator as previously described (Woodbury and Davenport, 1952). Corneal stimulation (60 Hz, 0.2 sec, varying current intensity) induced a sequence of behaviors in rats that started with tonic forelimb extension, followed by hindlimb flexion and terminated in full tonic hindlimb extension (180 degrees to the torso) (Toman, 1951; see Peterson, 1998; see White et al., 2002). The 6-Hz partial psychomotor seizure threshold was assessed with a Grass S48 stimulator, which delivered current at a lower frequency and longer duration (6 Hz, 3 sec, varying intensities). The phenotypes of the 6-Hz partial limbic seizure include rhythmic face movements, forelimb clonus, dorsal neck flexion, rearing and falling, elevated tail (Straub tail) and/or transient gait wobbliness/ataxia (Barton et al., 2001). After stimulation, the rat was quickly rolled onto its side so that the evoked convulsion could be observed clearly by the investigator.

The seizure threshold was determined according to the "staircase" procedure as previously described (Swinyard 1972) wherein the individual animal's response to a given stimulus intensity determines the subsequent current intensity to be tested in the next animal. Based on these data, the convulsive current (CC) curve was generated. The median convulsive current (CC50) required to induce the desired seizure endpoint in 50% of the rats tested was estimated and compared between groups using Probit analysis (Finney, 1971) with the statistical software Minitab (State College, PA). The difference was considered significant at $p < 0.05$. 
Toxicity

The effect of METH and dopaminergic modulators on an individual rat's motor and neurological function was assessed using a battery of tests (gait and stance test, placing response test and muscle tone test). Motor function was considered impaired if rats failed in two of these three tests.

Rat rectal temperature monitoring

Rat rectal temperatures were measured every half hour during the experiments with a digital readout thermocouple (BAT-12, Physitemp Instruments Inc, Clifton, NJ). The last measurement was at the time point just before the seizure threshold test. The temperature probe was dipped into mineral oil for lubrication before being inserted 3 cm into the rat rectum. When the temperature reading on the digital panel of thermometer stabilized (usually within 15 s), it was recorded as the rectal temperature of that rat. After each experiment, the probe was cleaned with 70% alcohol.

Statistical analysis

The statistical methods employed in these studies included Probit analysis for CC50 estimation and one-way ANOVA for rat temperature comparison. Differences were considered significant at $p < 0.05$.

Results

The effects of METH, SCH-23390 and eticlopride on rat rectal temperatures

Rats were randomly divided into four groups which were "saline-treated", "METH-treated", "antagonist-treated" and "antagonist plus METH-treated". In the "antagonist plus METH-treated" group, the dopamine D-1 antagonist SCH-23390 (0.5 mg/kg, i.p.) or the D-2 antagonist eticlopride (0.5 mg/kg, i.p.) was administered 30 min or 40 min prior to METH (15 mg/kg, s.c), respectively. The other three groups
were injected with the same doses of METH, antagonists or equivalent amount of saline. Seizure threshold was assessed 1 h after drug treatment. As shown in Fig. 3.1, there was no significant difference in the rat rectal temperatures between groups ($p > 0.05$). Therefore, the changes observed in seizure threshold are thought to be independent of hyperthermia, which could alter seizure susceptibility (Morimoto et al., 1992).

The effect of D-1 receptor blockade on METH-induced increase of threshold for tonic hindlimb extension

The threshold for tonic hindlimb extension was markedly increased 1 h after METH treatment (15 mg/kg, s.c). As shown in Fig. 3.2, the CC50 was increased from 36.1 ± 12 mA in saline-treated animals to 98.5 ± 3.4 mA in METH-treated animals ($p < 0.01$). The blockade of the D-1 receptor by SCH-23390 (0.5 mg/kg, i.p.) resulted in a synergistic effect with METH. For example, the CC50 for tonic hindlimb extension seizure was increased from 98.5 ± 3.4 mA to 551.0 ± 41.7 mA by SCH-23390 (Fig. 3.2). However, treatment with SCH-23390 alone did not affect seizure threshold (Fig. 3.2).

D-1 receptor blockade potentiates the METH-induced proconvulsant effect in the 6-Hz psychomotor seizure threshold test

In contrast to elevating the threshold for tonic hindlimb extension, a single METH treatment (15 mg/kg, s.c.) decreased the seizure threshold in the 6-Hz test. The CC50 was reduced from 83.4 ± 2.5 mA to 69.0 ± 2.3 mA after a single dose of METH (15 mg/kg, s.c.) ($p < 0.01$, Fig. 3.3). Rats pretreated with SCH-23390 (0.5 mg/kg, i.p.) displayed an even lower threshold (CC50: 57.7 ± 1.4 mA) when challenged with METH, whereas SCH-23390 alone did not have any effect on the 6-Hz seizure threshold (Fig. 3.3).
Figure 3.1. The effect of METH (15 mg/kg, s.c), SCH-23390 (0.5 mg/kg, i.p.) and eticlopride (0.5 mg/kg, i.p.) on rat rectal temperatures. Temperatures were recorded every 0.5 h before seizure threshold testing with a digital readout thermocouple. Rats were divided into four groups: "saline-treated" , "METH-treated" , "antagonist-treated" and "antagonist plus METH-treated" . The dopaminergic D-1 antagonist SCH-23390 (0.5 mg/kg) and D-2 antagonist eticlopride (0.05, 0.5 or 5 mg/kg) were administered i.p. 30 min and 40 min prior to METH injection, respectively. The seizure threshold was tested 1 h after drug treatment. (A) The effects of METH (15 mg/kg, s.c.) and SCH-23390 (0.5 mg/kg, i.p.) on rat rectal temperatures. There was no significant difference in the rat rectal temperature between "saline alone" (n = 12), "METH alone" (n = 12), "SCH-23390 alone" (n = 24) and "SCH-23390 plus METH" (n = 29) groups (p > 0.05, ANOVA). (B) The effects of METH (15 mg/kg, s.c.) and eticlopride (0.5 mg/kg, i.p.) on rat rectal temperatures. There was no significant difference in the rat rectal temperature between "saline alone" (n = 12), "METH alone" (n = 12), "eticlopride alone" (n = 24) and "eticlopride plus METH" (n = 24) groups (p > 0.05, ANOVA). Error bars represent S.E.M.
Figure 3.2. SCH-23390 treatment (0.5 mg/kg, i.p.) produced a synergistic interaction with METH (15 mg/kg, s.c.) in elevating the tonic hindlimb extension seizure threshold. A single METH injection (15 mg/kg, s.c.) significantly elevated the tonic hindlimb extension seizure threshold. The CC50 increased from 36.1 ± 1.2 mA in saline group to 98.5 ± 3.4 mA in METH group (p < 0.01). The selective D-1 antagonist SCH-23390 alone did not affect seizure threshold. However, blockade of D-1 receptors by SCH-23390 resulted in an enhancement of METH's ability to elevate the tonic hindlimb extension threshold (CC50: 98.5 ± 3.4 mA and 551.0 ± 41.7 mA in 'METH alone' and 'SCH-23390 plus METH' groups, respectively). The CC50 values and S.E.M. for individual groups are listed in legends. Probit analysis was conducted with the statistical program Minitab (State College, PA). **/? < 0.01; n = 14, 10, 16, 16 for the "saline", "METH", "SCH-23390" and "SCH-23390 plus METH" groups, respectively.
Figure 3.3. Blockade of D-l receptor by SCH-23390 (0.5 mg/kg, i.p.) produced a synergistic interaction with METH in the 6-Hz psychomotor seizure threshold test. A single METH injection (15 mg/kg, s.c.) lowered the 6-Hz seizure threshold. The CC50 was reduced from 83.4 ± 2.5 mA in saline group to 69.0 ± 2.3 mA in METH group (p < 0.01). The selective D-l antagonist SCH-23390 alone did not affect seizure threshold. However, blockade of D-l receptor activation by SCH-23390 (0.5 mg/kg, i.p.) 30 mins prior to METH resulted in an even lower seizure threshold (CC50: 69.0 ± 2.3 mA and 57.7 ± 1.4 mA, in 'METH alone' and 'SCH-23390 plus METH' groups, respectively). The CC50 values and S.E.M. for individual groups are listed in legends. Probit analysis; ** p < 0.01; n = 12, 10, 12, 17 for the "saline" , "METH" , "SCH-23390" and "SCH-23390 plus METH" groups, respectively.
Partial Psychomotor Seizure

CC50 (mA)

- 83.4 ± 2.5 mA
- 69.0 ± 2.3 mA
- 78.4 ± 2.4 mA
- 57.7 ± 1.4 mA
The D-2 receptor antagonist eticlopride blocks the METH-induced elevation of tonic hindlimb extension seizure threshold

METH (15 mg/kg, s.c.) elevated the tonic hindlimb extension threshold (CC50: 31.5 ± 2.9 mA and 105.4 ± 6.4 mA, in saline- and METH-treated groups, respectively) (Fig. 3.4). This increase was partially blocked by a single dose of eticlopride (0.5 mg/kg, i.p.) administered 40 mins prior to the METH challenge (Fig. 3.4). The CC50 in "eticlopride plus METH" group was reduced to 58.3 ± 3.8 mA compared with 105.4 ± 6.4 mA in METH-treated group, but was still higher than that of saline-treated group (31.5 ± 2.9 mA) (Fig. 3.4). The selective D-2 antagonist eticlopride (0.5 mg/kg, i.p.) alone did not affect the tonic hindlimb extension threshold (Fig. 3.4).

Eticlopride blocks the METH-induced decrease of 6-Hz psychomotor seizure threshold

As noted before, METH lowers the 6-Hz psychomotor seizure threshold. A single dose of METH (15 mg/kg, s.c.) decreased the CC50 for 6-Hz psychomotor seizure from 88.4 ± 4.4 mA to 67.3 ± 4.6 mA (Fig. 3.5). Treatment with eticlopride (0.5 mg/kg, i.p.) 40 mins prior to METH administration blocked the METH-induced decrease of 6-Hz psychomotor seizure threshold and the CC50 was brought back to 95.3 ± 6.4 mA (Fig. 3.5). Again, eticlopride itself did not change the seizure threshold (Fig. 3.5).

The effect of dopamine D-1 and D-2 receptor blockade on METH-induced elevation of tonic hindlimb extension seizure threshold

As shown in Fig. 3.6, SCH-23390 (0.5 mg/kg, i.p.) potentiated METH's ability to elevate the seizure threshold for tonic hindlimb extension. Eticlopride blocked this SCH-23390/METH synergism in a dose-dependent manner (Fig. 3.6). The CC50 in the "saline alone", "METH alone", "SCH-23390 plus METH", "eticlopride (0.05 mg/kg) plus SCH-23390 plus METH", "eticlopride (0.5 mg/kg) plus SCH-23390 plus METH"
Figure 3.4. Eticlopride treatment (0.5 mg/kg, i.p.) blocked the METH-induced elevation of tonic hindlimb extension seizure threshold. A single METH injection (15 mg/kg, s.c.) elevated the threshold for tonic hindlimb extension and increased the CC<sub>50</sub> from 31.5 ± 2.9 mA in saline-treated group to 105.4 ± 6.4 mA in METH-treated group (p < 0.01). The selective D-2 antagonist eticlopride alone did not affect seizure threshold. However, eticlopride (0.5 mg/kg, i.p.) administered 40 mins before METH challenge attenuated the METH-induced elevation of tonic hindlimb extension threshold and reduced the CC50 to 58.3 ± 3.8 mA. The CC50 values and S.E.M. for individual groups are listed in legends. Probit analysis; ** p < 0.01; n = 8, 23, 16, 16 for "saline" , "METH" , "eticlopride" and "eticlopride plus METH" groups, respectively.
Tonic Hindlimb Extension

- Saline: 25.0 ± 2.5 mA
- METH: 105.4 ± 6.4 mA
- Eticlopride: 31.5 ± 2.9 mA
- Eticlopride + METH: 58.3 ± 3.8 mA
**Figure 3.5.** Eticlopride treatment (0.5 mg/kg, i.p.) blocked the METH-induced decrease in the 6-Hz psychomotor seizure threshold. A single METH injection (15 mg/kg, s.c.) lowered the threshold for 6-Hz psychomotor seizure and decreased the CC₅₀ from 88.4 ± 4.4 mA to 67.3 ± 4.6 mA (p < 0.05). The selective D-2 antagonist eticlopride alone did not affect seizure threshold. However, treatment with eticlopride (0.5 mg/kg, i.p.) 40 min before METH challenge (15 mg/kg, s.c.) blocked the METH-induced decrease of 6-Hz seizure threshold and brought the CC₅₀ back to 95.3 ± 6.4 mA. The CC₅₀ values and S.E.M. for individual groups are listed in legends. Probit analysis; *p < 0.05; n = 18, 11, 13, 11 for "saline" , "METH" , "eticlopride" and "eticlopride plus METH" groups, respectively.
Partial Psychomotor Seizure

CC50 (mA)

- 88.4 ± 4.4 mA
- 67.3 ± 4.6 mA
- 86.7 ± 5.4 mA
- 95.3 ± 6.4 mA
Figure 3.6. The effect of dopamine D-1 and D-2 receptor blockade on METH-induced elevation of tonic hindlimb extension seizure threshold. A single dose of METH (15 mg/kg, s.c.) elevated the tonic hindlimb extension threshold. The D-1 antagonist SCH-23390 (0.5 mg/kg, i.p.) produced a synergistic interaction with METH. The D-2 antagonist eticlopride (0.05 mg/kg, 0.5 mg/kg, 5 mg/kg; i.p.) blocked the SCH-23390/METH synergy in a dose-dependent manner. The CC50 values and S.E.M. for individual groups are listed in legends. ** p < 0.01 (two-way), *p < 0.05 (two-way), f/? < 0.05 (one-way); Probit analysis; n = 8, 7, 20, 14, 15, 7 for "saline", "METH", "SCH-23390 plus METH", "eticlopride (0.05 mg/kg) plus SCH-23390 plus METH", "eticlopride (0.5 mg/kg) plus SCH-23390 plus METH" and "eticlopride (5 mg/kg) plus SCH-23390 plus METH" groups, respectively.
The effect of D-2 receptor activation on tonic hindlimb extension and 6-Hz partial psychomotor seizure threshold

As shown in Fig. 3.2-3.5, SCH-23390 and eticlopride displayed synergistic and antagonistic effects with METH in the seizure threshold studies conducted, respectively. As such, the selective D-2 agonist quinpirole was tested for its ability to modulate tonic hindlimb extension threshold and 6-Hz psychomotor seizure threshold. The results demonstrate that a single administration of quinpirole (20 mg/kg, i.p.) decreased the CCsof for both tonic hindlimb extension (from 27.1 ± 2.3 mA to 17.8 ± 1.9 mA) and 6-Hz psychomotor seizures (from 91.9 ± 21.6 mA to 60.6 ± 15 mA) (Fig. 3.7).

Discussion

The results outlined in the present investigation demonstrate that the effects of METH on electroconvulsant seizure threshold can be enhanced or blocked by applying selective dopamine receptor antagonists. The results suggest that dopamine receptor modulation is involved in METH's ability to modify seizure threshold. Furthermore, they demonstrate that D-1 and D-2 receptors play opposite roles in modifying seizure susceptibility. The lack of any significant temperature changes or notable motor/neurological toxicity following METH administration rules out hyperthermia and toxicity as an influence on the seizure threshold changes that were observed.

As shown in Fig. 3.2, METH exerts an anticonvulsant effect on the tonic hindlimb extension seizure, which is one form of generalized seizures. Previous
Figure 3.7. The effect of the dopamine D-2 agonist quinpirole (20 mg/kg, i.p.) on tonic hindlimb extension and 6-Hz psychomotor seizure thresholds. A single dose of quinpirole (20 mg/kg, i.p.) was administered to rats. No notable motor or neurological toxicity was observed. One hour later, the threshold for tonic hindlimb extension and 6-Hz psychomotor seizures were determined. (A) Quinpirole decreased the threshold for tonic hindlimb extension. The CC50 was reduced from 27.1 ± 2.3 mA in saline-treated group to 17.8 ± 1.9 mA in quinpirole-treated group.* p < 0.05; Probit analysis; n = 11, 12 for saline- and quinpirole-treated groups, respectively. (B) Quinpirole decreased the 6-Hz psychomotor seizure threshold. The CC50 was significantly lower in the quinpirole-treated group (60.6 ± 1.5 mA) than in the control group (91.9 ± 2.6 mA). ** p < 0.01; Probit analysis; n = 17 in both groups. The CC50 values and S.E.M. for individual groups are listed in legends.
A

**Tonic Hindlimb Extension**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CC50 (mA)</th>
</tr>
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<tbody>
<tr>
<td>Saline</td>
<td>27.1 ± 2.3 mA</td>
</tr>
<tr>
<td>Quinpirole</td>
<td>17.8 ± 1.9 mA</td>
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B

**Partial Psychomotor Seizure**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CC50 (mA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>91.9 ± 2.6 mA</td>
</tr>
<tr>
<td>Quinpirole</td>
<td>60.6 ± 1.5 mA</td>
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studies suggest that generalized seizures originate from brainstem structures including the caudal deep layers of the superior colliculus, the adjacent intercollicular nucleus and underlying reticular formation (Browning, 1985; Redgrave et al., 1992a&b; Shehab et al., 1995a,b&c), which is designated as the dorsal midbrain anticonvulsant zone (DMAZ) (Shehab et al, 2005). The substantia nigra pars reticulata (SNr) contains mainly GABAergic neurons with high spontaneous firing rates (MacLeod et al., 1980; Chevalier et al, 1985) whose axons project to the DMAZ (Niijima and Yoshida, 1982; Giolli et al., 1985; Garant and Gale, 1987). Experimental evidence has demonstrated that the SNr is a critical site involved in the propagation of generalized convulsions (Iadarola and Gale, 1982; Waszczak et al., 1986; Garant and Gale, 1987; Veliskova et al., 2002; Veliskova and Moshe, 2006): a decreased activity of SNr neurons results in the prevention of seizure generalization; whereas an increased SNr activity lowers seizure threshold (Iadarola and Gale, 1982; also see Veliskova and Moshe, 2006). For example, microinjection of the direct GABA receptor agonist, muscimol, into the midbrain attenuated tonic hindlimb extension seizures (Iadarola and Gale, 1982). The anticonvulsant role of SNr inhibition is thought to be mediated by the disinhibition of its output structures such as superior colliculus (SC) and pedunculopontine tegmental nucleus (PPT) (Bolam et al., 2000), which leads to the prevention of seizure generalization (Dean and Gale, 1989; Depaulis et al., 1990; Redgrave et al., 1992b; Shehab et al., 2005; Fig. 3.8).

Previous studies have suggested that the role of dopamine transmission in seizure mechanisms depends on how it affects the major striatal efferent GABAergic pathways terminating in the SNr or globus pallidus (Iadarola and Gale, 1982; Fariello et al., 1987; Patel et al., 1986; Turski et al., 1988; Ogren and Pakh, 1993). There are direct (striatonigral) and indirect (striatopallidosubthalamic-nigral) pathways
Figure 3.8. Diagram of the afferents and efferents of substantia nigra pars reticulata (SNr). The SNr receives input from striatum from direct (striato-nigral) and indirect (striato-pallido-subthalamic-nigral) pathways. The SNr sends out GABAergic projections to its output structures including the thalamus, superior colliculus (SC) and pedunculopontine tegmental nucleus (PPT). The D-1 receptor activation in the SNr and D-2 activation in the striatum excites and inhibits the SNr, respectively. GPe: globus pallidus external; GPi: globus pallidus internal; PPT: pedunculopontine tegmental nucleus; SC: superior colliculus; SNc: substantia nigra pars compacta; SNr: substantia nigra pars reticulata; STN: subthalamic nucleus.
connecting striatum to the SNr (for reviews, see Albin et al., 1989; Alexander and Crutcher, 1990; Bolam et al., 2000; Fig. 3.8). Activation of the direct pathway, modulated by the D-1 receptor, increases GABA-mediated inhibition on the SNr neurons, while the indirect pathway, which sends excitatory glutamatergic projections to the SNr from the subthalamic nucleus (STN), is modulated by D-2 receptors (Gerfen et al., 1990, also see Surmeier et al., 2007). METH treatment increases striatal extracellular DA levels (O'Dell et al., 1991; Weihmuller et al., 1991). The striatal D-2 receptor activation inhibits the striato-pallidal neurons (for review, see Surmeier et al., 2007) and the SNr following the indirect pathway (for reviews, see Albin et al., 1989; Alexander and Crutcher, 1990; Bolam et al., 2000), which leads to the prevention of generalized seizures based on the discussion above. The antagonistic effect between eticlopride and METH is hypothesized to be due to the blockade of striatal D-2 receptors and the SNr inhibition. In contrast, the striatal D-1 receptor does not seem to play an important role in seizure susceptibility, which is supported by other research demonstrating that the intrastriatal injection of D-1 agonist SKF-38393 does not affect pilocarpine seizures (Turski et al., 1990).

However, there are abundant D-1 receptors located on the terminals of GABAergic striatonigral neurons in the SNr (Porceddu et al., 1986; Trevitt et al., 2004; Fig. 3.8). The SCH-23390/METH synergism is speculated to be mediated by SCH-23390-induced blockade of D-1 receptors located on the GABAergic terminals of striatonigral neurons. Dopamine (DA) can be dendritically released from the SNC (substantia nigra pars compacta) to SNr (for review, see Cheramy et al., 1981; Heeringa and Abercrombie, 1995). Electrophysiological studies indicate that DA in the SNr attenuates the striatonigral inhibition and activates SNr through presynaptic D-1 receptors (Matthews and German, 1986). Therefore, treatment with SCH-23390
can cause the inhibition of SNr by blocking its presynaptic D-1 receptors, which results in prevention of seizure generalization. Microinjection studies support the above hypothesis. For example, Turski and colleagues have demonstrated that dopamine alters seizure threshold via D-1 pathway in the SNr (rather than the striatum) and the D-2 pathway in the striatum (Turski et al., 1990). Moreover, the D-1 (SCH-23390) and D-2 (haloperidol) antagonists display anticonvulsant and proconvulsant effects in pilocarpine-induced generalized seizure, respectively (Turski et al, 1990).

In contrast to elevating the tonic hindlimb extension seizure threshold, METH lowered the partial limbic seizure threshold tested in the 6-Hz model. Previous research has suggested that the 6-Hz psychomotor seizure mainly activates limbic brain structures (Barton et al., 2001). The mediodorsal thalamus has been shown to play an important role in the development of limbic motor seizure (Cassidy and Gale, 1998). As discussed above, METH treatment produces inhibition of SNr by modulating the dopaminergic system, which results in the disinhibition of its output targets including mediodorsal thalamus. It has been demonstrated that enhanced excitation of mediodorsal thalamus leads to decreased limbic motor seizure threshold (Cassidy and Gale, 1998). Therefore, METH exerts its proconvulsant effect observed in the 6-Hz model. The approach of single extracellular unit recording (Boraud et al., 2002) could be employed to test the proposed neuroanatomical mechanisms above by assessing the excitability of the SNr and mediodorsal thalamus.

In addition to the dopaminergic antagonist studies, we evaluated the effect of D-2 agonist quinpirole (20 mg/kg, i.p.) on the tonic hindlimb extension and 6-Hz psychomotor seizure threshold. Based on the discussion above, it is hypothesized that quinpirole would exert anticonvulsant and proconvulsant effects in the tonic hindlimb
extension and 6-Hz psychomotor models, respectively. The results indicated that quinpirole decreases the 6-Hz threshold as expected. However, it did not elevate the tonic hindlimb extension seizure threshold. The reason could be that the agonist quinpirole, applied at the dose of 20 mg/kg, may affect other systems like norepinephrine (NE) (Fuller and Hemrick-Luecke, 2007), which modifies the seizure threshold as well (see Giorgi et al., 2004). Moreover, our studies found that lower doses of quinpirole (2 mg/kg, i.p.) did not affect either tonic hindlimb extension or 6-Hz psychomotor seizure threshold, which was assessed 1 h after quinpirole treatment (data not shown).

In addition to dopaminergic neurotransmission, other mechanisms may contribute to METH's effect on electroconvulsant seizure threshold. For example, it is known that serotonin (5-HT) is released by acute METH challenge (Berger et al., 1992). The neurotransmitter 5-HT has been demonstrated to modify seizure susceptibility in various seizure models (Lerner-Natoli, 1987). The elevation of 5-HT levels inhibits seizure generation, whereas a decrease in 5-HT function increases seizure susceptibility (see Peterson and Alberson, 1982; Waterhouse, 1986). For instance, fluoxetine, a 5-HT reuptake inhibitor, was reported to increase the dose of picrotoxin required to induce tonic hindlimb extension in mice (Pericic et al, 2005a). Moreover, stimulation of 5-HT 1A receptor using its selective agonist 8-OH-DPAT [(+/-)-8-hydroxy-2-(di-n-propylamino) tetralin] caused an increase of the picrotoxin doses required to produce running/bouncing clonus and tonic hindlimb extension and in mice (Pericic et al., 2005b). A recent study by Stean has shown that stimulation of postsynaptic 5-HT IB receptors inhibits electroshock-induced seizure spread in rats (Stean et al., 2005). Therefore, it is very likely that activation of the serotonergic system also contributes to the anticonvulsant effect of METH in the tonic hindlimb
extension seizure threshold test.

In conclusion, the results in this investigation have provided evidence for an important role of dopaminergic neurotransmission in mediating METH's ability to modulate seizure threshold. It is likely that dopaminergic activation in the striatum and SNr affects seizure threshold by modifying the excitation state of SNr, which is the gating structure mediating seizure spread. Specifically, dopaminergic D-2 receptor activation and D-1 activity may mediate opposing effects on the SNr activity and thus seizure susceptibility. Other neurotransmitters such as 5-HT may also contribute to the anticonvulsant effect of METH on the tonic hindlimb extension seizure.
References


Hanson GR, Jensen M, Johnson M, White HS (1999) Distinct features of seizures


Slamberova R (2005) Flurothyl seizures susceptibility is increased in prenatally


CHAPTER 4

THE EFFECT OF MULTIPLE METHAMPHETAMINE TREATMENT
ON RAT AMYGDALA KINDLING ACQUISITION
AND BRAIN MONOAMINE LEVELS

Introduction

Epilepsy is a chronic neurological disorder characterized by recurrent spontaneous seizures. Epileptogenesis is defined as a sequence of events that converts a normal neuronal network into a hyperexcitable network which produces recurrent spontaneous seizures (see Lowenstein, 1996; Dudek and Clark, 2004; see Dichter, 2006). Genetic defects can lead to epileptogenesis (see Lerche et al., 2005). In addition, acquired or symptomatic epilepsy can result from specific insults to the brain such as head trauma, stroke and infection. Many of these insults are thought to exert their pro-epileptogenic effect by enhancing glutamatergic neurotransmission (Jin et al., 2006), decreasing GABA-mediated inhibition (Sloviter, 1991), altering monoaminergic modulation (see Kobayashi and Mori, 1977; Teskey et al., 2004), increasing neuronal death (see DeLorenzo and Sun, 2006) or network reorganization (Sutula et al., 1989; Represa et al., 1993). Moreover, nonsynaptic (Naus et al., 1991) and other mechanisms (for reviews, see Lowenstein, 1996; Acharya, 2002) could also contribute to the process of epileptogenesis.

The kindling phenomenon, first discovered by Graham Goddard in 1967 (Goddard, 1967), is described as the progressive intensification of both behavioral and electrographic seizures as a result of repeated, subthreshold electrical stimulations to
particular brain structures (Goddard et al., 1969). Consecutive kindling stimulations result in spontaneous seizures (Michalakis et al., 1998; Potschka et al., 2000). Therefore, the kindling phenomenon has been utilized as a model for complex partial seizure with secondary generalization and is a model of brain plasticity associated with kindling-induced epileptogenesis (see Engel and Shewmon, 1991; see Bertram, 2007). Since its discovery, numerous researchers have used the kindling model to study the mechanisms underlying the development of a hyperexcitable network. This effort is of potential importance in that it can help to identify molecular targets and the ultimate development of novel therapies for the treatment and prevention of epilepsy.

Methamphetamine (METH), a schedule II drug, can cause convulsions in patients following acute intoxication (Lan et al., 1998). However, it is not known whether chronic METH use enhances the epileptogenic process. In 1992, one research group reported that chronic METH treatment (6 mg/kg/day, 14 days) significantly and selectively decreased the number of stimulations required to trigger an epileptic afterdischarge in the first two hippocampal kindling stimulations (Minabe and Emori, 1992). The present study further assessed METH's influence on epileptogenesis in the rat amygdala kindling model. The outcome measures evaluated in this study included assessing the effect of METH on afterdischarge threshold (ADT), kindling acquisition rate and afterdischarge duration (ADD).

In addition, the effect of METH on brain monoamines was investigated. It is known that a single large dose or multiple smaller doses of METH cause striatal dopamine and hippocampal serotonin reduction and even depletion (Kogan et al., 1976; Seiden et al., 1976; Ricautre et al., 1980; Marek et al., 1990; Fukumura et al., 1998). This METH-induced monoaminergic neurotoxicity is attributed to multiple
factors including hyperthermia (Albers and Sonsalla, 1995; see also Riddle et al., 2006). Moreover, it is age-dependent. For example, METH is less toxic in neonatal and adolescent rats compared to adult rats (Lucot et al., 1982; Truong et al., 2005; Rau et al., 2006). Brain monoamines play an important role in modulating kindling development (Arnold et al., 1973; Corcoran et al., 1974; Callaghan and Schwark, 1979; Corcoran and Mason, 1980; McIntyre et al., 1979; Teskey et al., 2004). For example, serotonin (5-HT) retards kindling development (Lerner-Natoli, 1987); norepinephrine (NE) has been reported to be involved in the generation, development or maintenance of seizure activity in animal kindling (see Corcoran, 1981). Therefore, measurement of monoamine (DA, 5-HT and NE) levels in amygdala, hippocampus and striatum helps to delineate the neurochemical mechanisms underlying METH's effect on amygdala kindling acquisition.

Methods

Animals

Male Sprague-Dawley rats were purchased from Charles River laboratories (Wilmington, MA) and used for all experiments. All rats were housed in a temperature (22°C) and light (12-h light/dark cycle) controlled environment maintained by the University of Utah Comparative Medicine Center. Rats were allowed free access to food and water. All experiments were conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. All protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Utah.
Drugs and chemicals

(±)-Methamphetamine hydrochloride was provided by the National Institute on Drug Abuse (Rockville, MD) and doses were calculated as the free base. Ketamine hydrochloride (100 mg/kg, i.p., Abbott Laboratories, N. Chicago, IL) and xylazine (10 mg/kg, i.p., Sigma, St. Louis, MO) were administered for anesthesia before stereotaxic surgery. Reagents for HPLC-ECD (high performance liquid chromatography-electrochemical detector) were obtained from Sigma Chemical Co. (St. Louis, MO).

METH administration and rat rectal temperature measurement

Male Sprague-Dawley rats (260-280 grams) were randomly divided into two groups. One group received four METH injections (4 x 10 mg/kg, s.c.) with a 2-h interval (9am, 11am, 1pm and 3pm), and the other group was administered an equivalent amount of saline and served as control. Rat rectal temperatures were measured every 0.5 h with a digital readout thermocouple (BAT-12, Microprobe Thermometer, Physitemp Instruments, Inc, Clifton, NJ). The temperature probe coated with mineral oil was inserted 3 cm into the rat rectum. When the temperature reading stabilized (within approximately 15 sec), it was recorded as the rectal temperature for that rat.

Stereotaxic surgery

Three days after METH/saline treatment, stereotaxic surgeries were conducted in rats under ketamine/xylazine anesthesia. A bipolar stimulating-recording electrode (Plastics One, Inc., Roanoke, VA) was implanted into the right basolateral amygdala (coordinates: AP, - 0.22 cm; ML, - 0.47 cm; DV, - 0.87 cm, relative to bregma) as previously described (Loscher and Honack, 1995; Paxinos and Watson, 1998). Three
anchor screws were adhered to the skull with dental acrylic cement to help immobilize the electrode. Animals were allowed one week of recovery prior to initiating the kindling protocol.

Kindling

Rats were moved to kindling cages where their implanted electrodes were connected to a Grass model SD9 stimulator (Astro-Med; Grass Instrument Division, West Warwick, RI). The kindling stimulus consisted of a 1-sec train of biphasic square-wave pulses. Electroencephalograph (EEG) was digitally recorded using a computer and MP100 Manager software (version 3.5.3, Biopac Systems, Inc.). On the first day, the afterdischarge threshold (ADT) was assessed by stimulating the amygdala with increasing stimulus intensity beginning with 40 uA and increasing by 20 uA increments until an afterdischarge (a discharge of spikes for the duration of at least 3 sec) was first observed. This current intensity was recorded as the ADT and reflective of each animal's electrographic seizure threshold. Starting from the next day, each rat was stimulated at its individual ADT once per day until a fully-kindled status was reached. Fully-kindled status was defined as three consecutive stage 4/5 seizures by Racine scale: "0", no seizure; "1", mild head nodding with or without jaw chomping; "2", 5-10 sec of unilateral forelimb clonus; "3", forelimb clonus; "4", rearing with forelimb clonus; "5", rearing and falling with forelimb clonus (Racine, 1972). The behavioral seizure score and afterdischarge duration (ADD) after each kindling stimulation were recorded for individual rats.

Monoamine content measurement by high performance liquid chromatography-electrochemical detector (HPLC-ECD)

Rats were decapitated after reaching fully kindled status and their brains were rapidly removed and placed on dry ice. The striatum and hippocampus were dissected...
directly from half of the brain as described by Johnson-Davis (Johnson-Davis et al., 2003). The other half of the brain was used to obtain coronal slices. The amygdala was dissected from the slice by cutting along the upper and lateral borders of the amygdala (Honkanen, 1999). Dissected tissues were weighed and sonicated in tissue buffer [0.05 M sodium phosphate/0.03 M citric acid with 15% methanol (vol/vol), pH 2.5]. After centrifuging at 18,800 * g for 15 mins at 4°C, the supernatant was collected and centrifuged at 18,800 * g for 10 mins at 4°C. Twenty microliters of supernatant was injected onto a HPLC system (Dynamax AI-200 Autosampler and SD-200 pump; Varian, Walnut, CA), which is coupled to an electrochemical detector (Eox = + 0.70 V; Varian Star 9080). Monoamines were separated by a Whatman PartiSphere C-18 column (250 x 4.6 mm, 5um; Whatman, Clifton, NJ). The mobile phase (pH 2.87) included methanol (23% vol/vol), sodium octyl sulfate (0.03% wt/vol), ethylenediaminetetraacetic acid (EDTA) (0.1mM), sodium phosphate dibasic (0.05 M), and citric acid (0.03 M). The flow rate was 1 ml/min. Protein content of the tissues was detected by a Bicinchoninic Acid Protein Assay Kit (Sigma, St. Louis, MO). Monoamine levels were normalized to protein content per sample.

Statistical analysis

Nonpaired Student's t-test was employed for the comparison of rat rectal temperatures, ADTs, ADDs and the numbers of stimulations to reach stage 3 or stage 4/5 seizures between the saline- and METH-treated groups. Fisher's exact test was applied for the intergroup comparison of cumulative percentage of rats reaching stage 3 or stage 4/5. Differences were considered significant at/? < 0.05.
Results

The effect of multiple METH treatment on rat rectal temperature

Hyperthermia contributes to METH-induced neurotoxicity (Albers and Sonsalla, 1995). Rats weighing 260-280 g were administered four doses of METH (4 x 10 mg/kg, s.c.) or saline. The rat rectal temperatures were recorded every 0.5 h. As shown in Fig. 4.1 and consistent with previous reports (Albers and Sonsalla, 1995; Baucum et al., 2004), multiple METH treatment caused significant hyperthermia ($p < 0.05$). After the second METH dose, the average core temperature of rats was observed to increase from 37.3°C to 38.8°C. This is in contrast to the saline-treated rats whose highest temperature was 37.3°C ($p < 0.05$). The ambient temperature was constant at 23°C.

The effect of multiple METH treatment on afterdischarge threshold (ADT)

One day prior to kindling, the ADT was assessed by stimulating the amygdala with increasing stimulus intensity beginning with 40 uA and increasing by 20 uA increments until an afterdischarge (a discharge of spikes for the duration of more than 3 sec) was first observed. There was no significant difference in ADT between the METH- and saline-treated groups, which were 140.0 ± 14.4 uA (mean ± S.E.M., n = 14) and 158.2 ± 19.5 uA (n = 11), respectively ($p > 0.05$, Student’s t-test; Fig. 4.2).

The effect of multiple METH treatment on amygdala kindling acquisition rate

The kindling acquisition rate was defined by the number of stimulations required for rats to develop seizures of a certain stage according to Racine scale (Racine, 1972). To reach stage 3, METH-treated rats required 5.0 ± 0.3 stimuli while those in the saline-treated group required significantly more stimuli: 8.3 ± 0.9 ($p < 0.01$, Student’s t-test; Fig. 4.3A). Likewise, the saline-treated rats received more
Figure 4.1. Effect of multiple METH treatment (4 x 10 mg/kg, s.c.) on rat rectal temperatures. Rats (260-280 g) were randomly divided into two groups: one group received four doses of METH (4 x 10 mg/kg, s.c.) while the other was administered saline and served as control. Rat rectal temperatures were recorded every 0.5 h using a digital readout thermocouple. The four arrows represent the time points of METH/saline treatment. * $p < 0.05$; Student's Mest; $n = 11, 14$; saline- and METH-treated groups, respectively. Error bars represent S.E.M.
Figure 4.2. Effect of multiple METH treatment (4 * 10 mg/kg, s.c.) on the afterdischarge threshold (ADT) of amygdala kindling. The rat amygdala was electrically stimulated by increasing current intensity until an afterdischarge (a discharge of spikes for a duration of at least 3 sees) was evoked, and this intensity was used as the ADT. There was no difference between the ADTs of METH- and saline-treated rats: 140.0 ± 14.4 uA (mean ± S.E.M., n = 14) and 158.2 ± 19.5 uA (n = 11), respectively (p > 0.05, Student's t-test).
stimuli (11.6 ± 1.2) than METH-treated ones (7.3 ± 0.6) to reach stage 4/5 ($p < 0.01$, Student's t-test; Fig. 4.3B). The data suggest that the kindling rate was significantly faster in the METH-treated group and thus support the conclusion that multiple METH treatment facilitates amygdala kindling acquisition.

As shown in Fig. 4.4, the cumulative percentage curve of METH-treated rats reaching stage 3 or stage 4/5 displayed a leftward shift compared with that of control. On the same kindling day, more rats in the METH-treated group than controls reached stage 3 or stage 4/5. For example, by kindling day 6, 100% of METH-treated rats had reached stage 3, whereas only 27.3% in saline group had developed a stage 3 seizure. Moreover, 100% vs. 40% (METH- vs. vehicle-treated) of rats had displayed a stage 4/5 seizure by the 11th kindling day (Fig. 4.4). These differences were significant at $p < 0.01$ tested by Fisher’s exact test.

The effect of multiple METH treatment on afterdischarge duration (ADD)

The afterdischarge duration (ADD) displayed a progressive increase in both METH- and saline-treated groups (Fig. 4.5). However, the average ADD of the METH-treated rats was longer than that of control animals for all stimulations except the 1st, 5th and 12th days (Fig. 4.5). These data suggest that METH-treatment enhances the duration of limbic seizures. However, the total ADD (the sum of each single ADD before reaching stage 3 or stage 4/5) was not different between saline- and METH-treated groups ($p > 0.05$) (Fig. 4.6).

The effect of multiple METH treatment on monoamine levels in the amygdala, hippocampus and striatum

Monoamine levels were detected using the approach of HPLC-ECD. Serotonin (5-HT) levels were decreased in the amygdala, hippocampus and striatum by 45%, 52% and 57%, respectively (Fig. 4.7). In addition, a significant dopamine (DA)
**Figure 4.3.** Effect of multiple METH treatment (4 x 10 mg/kg, s.c.) on amygdala kindling acquisition rate. Kindling stimulations were delivered to the rat basolateral amygdala once per day at its individual ADT. As kindling develops, the behavioral score and afterdischarge duration (ADD) displayed a progressive increase. Stimulations were stopped when rats reached a fully-kindled state defined as 3 consecutive stage 4/5 seizures. (A) The number of kindling stimulations required to reach stage 3: METH, 5.0 ± 0.3; saline, 8.3 ± 0.9. METH-treated group needed fewer stimuli to reach stage 3 than saline-treated group \( p < 0.01 \). (B) The number of kindling stimulations to reach stage 4/5: METH, 7.3 ± 0.6; saline, 11.6 ± 1.2. It took METH-treated rats fewer stimuli to reach stage 4/5 compared with controls \( p < 0.01 \). Values represent means ± S.E.M. \( ** \ p < 0.01 \); two-tailed, nonpaired Student’s Mest; n = 11, 14; saline- and METH-treated groups.
Figure 4.4. The cumulative percentage of rats reaching stage 3 or stage 4/5 seizures during amygdala kindling. (A) The cumulative percentage of rats reaching stage 3. An increasing percentage of rats in both METH- and saline-treated groups reached stage 3 as amygdala kindling developed. On the same kindling day, more rats in the METH-treated group than controls reached stage 3. For example, by kindling day 6, 100% of METH-treated rats had reached stage 3. In contrast, only 27.3% in saline-treated group had developed a stage 3 seizure ($p < 0.05$, Fisher's exact test). (B) The cumulative percentage of rats reaching stage 4/5. All (100%) METH-treated rats vs. 40% of saline-treated rats had reached stage 4/5 by the kindling day 11 ($p < 0.05$, Fisher's exact test).
Figure 4.5. The average afterdischarge duration (ADD) induced by amygdala kindling stimulation displayed a progressive increase over the kindling acquisition period. The kindling stimulation was delivered once per day. The ADD was measured after each kindling stimulation and averaged among rats of the same group. The kindling stimulation produced a longer ADD in METH-treated rats than in controls except on the 1st, 5th and 12th days. Values represent means ± S.E.M. * p < 0.05; one-tailed Student's t-test; n = 11, saline; 14, METH.
Figure 4.6. The total afterdischarge duration (ADD) to reach stage 3 or stage 4/5 seizures in amygdala kindling. Total ADD is calculated by summing all the single ADDs before a certain seizure stage is reached. (A) The total ADD to reach stage 3 was not significantly different between the two groups: METH, 56.31 ± 10.99 sec; saline, 76.82 ± 24.83 sec (p > 0.05, Student's t-test). (B) The total ADD to reach stage 4/5 showed no significant difference between groups: METH, 130.1 ± 20.83 sec; saline, 150.9 ± 33.62 sec (p > 0.05, Student's t-test). Values represent means ± S.E.M.
Figure 4.7. The effect of multiple METH treatment on brain serotonin (5-HT) levels in the amygdala, hippocampus and striatum. Fully kindled rats were decapitated and brain regions of amygdala, hippocampus and striatum were dissected out. Serotonin concentration was measured by high performance liquid chromatography-electrochemical detector (HPLC-ECD). (A) Amygdala 5-HT was reduced 46% after METH treatment (* p < 0.05, Student's t-test). (B) Hippocampal 5-HT was reduced 52% by METH treatment (**) p < 0.01). (C) Striatal 5-HT was depleted 57% by METH treatment (* p < 0.05). Error bars represent S.E.M.
A

Amygdala Serotonin

B

Hippocampal Serotonin

C

Striatal Serotonin
depletion (46%) was found in the striatum but not amygdala following multiple METH treatment (Fig. 4.8). In contrast to the striatum and amygdala, the hippocampus does not have detectable DA level. The norepinephrine (NE) system seemed to be resistant to METH challenge as there was no significant change in NE levels in the amygdala, hippocampus or striatum ($p > 0.05$, Fig. 4.9).

The age-dependence of METH's effect on hyperthermia, brain monoamine levels and amygdala kindling acquisition rate

It is known that hyperthermia contributes to METH-induced toxicity (Albers and Sonsalla, 1995; see also Riddle et al., 2006), which is age-dependent (Lucot et al., 1982; Truong et al., 2005; Rau et al., 2006). Rats weighing 260-280 g showed significant hyperthermia ($> 38.6^\circ$C) after multiple METH treatment (Fig. 4.10); the older rats weighing 280-300 g developed more severe hyperthermia ($> 39.0^\circ$C) and displayed a higher mortality rate after METH challenge (Fig. 4.10); in contrast, younger rats weighing 240-260 g displayed the least severe hyperthermia ($< 38.6^\circ$C, Fig. 4.10).

In an effort to determine whether the initial hyperthermic response contributed to a faster rate of kindling, we assessed the amygdala kindling rate in the younger rat group (240-260 g). There was no difference in the amygdala kindling rate between saline- and METH-treated rats (Fig. 4.11). The two groups required a similar number of stimulations to reach stage 3 or stage 4/5. Furthermore, there was no striatal DA or hippocampal 5-HT depletion in those METH-treated rats which displayed the least hyperthermic response ($< 38.6^\circ$C) to the initial METH challenge (Fig. 4.12). These results suggest a positive correlation between hyperthermia, METH-induced toxicity and amygdala kindling facilitation.
Figure 4.8. The effect of multiple METH treatment on brain dopamine (DA) levels in the amygdala and striatum. (A) Amygdala DA concentration was not changed after METH treatment ($p > 0.05$, Student's t-test). (B) Striatal DA was reduced 46\% by METH treatment (** $p < 0.01$). Hippocampus does not have detectable DA level. Error bars represent S.E.M.
Figure 4.9. The effect of multiple METH treatment on brain norepinephrine (NE) levels in the amygdala, hippocampus and striatum. (A) Amygdala NE level was not changed by METH treatment ($p > 0.05$, Student’s t-test). (B) Hippocampal NE concentration was unaltered after METH treatment ($p > 0.05$). (C) Striatal NE content was not different between saline- and METH-treated groups ($p > 0.05$). Error bars represent S.E.M.
Figure 4.10. The age-dependence of METH (4 x 10 mg/kg, s.c.)-induced hyperthermia. Rat rectal temperatures were recorded every 0.5 h before and after each METH/saline injection. The four arrows represent the time points of METH/saline treatment. Younger rats (240-260 g) treated with METH showed an attenuated hyperthermic response compared with older rats. Error bars represent S.E.M.
Figure 4.11. Effect of multiple METH treatment (4 x 10 mg/kg, s.c.) on amygdala kindling acquisition rate in the younger rats weighing 240-260 g. (A) The number of kindling stimulations required to reach stage 3 was not different between saline-treated (6.9 ±0.6, n = 8) and METH-treated (6.9 ±0.5, n = 10). (B) The number of kindling stimulations to reach stage 4/5 showed no difference between the two groups: saline, 8.6 ± 0.7; METH, 8.0 ± 0.6. Values represent means ± S.E.M. p > 0.05, Student's Mest.
Figure 4.12. The effect of multiple METH treatment on striatal DA and hippocampal 5-HT levels in the younger rats weighing 240-260 g. Striatal DA and hippocampal 5-HT concentrations were used as two markers representing METH-induced neurotoxicity. (A) Multiple METH treatment did not cause striatal DA depletion in the younger rats ($p > 0.05$, Student's t-test). (B) Multiple METH treatment did not deplete hippocampal 5-HT in the younger rats ($p > 0.05$). Error bars represent S.E.M.
A

**Striatal Dopamine**

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<th>ng / mg protein</th>
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<td>Saline</td>
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<td>METH</td>
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B

**Hippocampal Serotonin**

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<tr>
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<td>12.5</td>
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Discussion

Kindling has long been utilized as a model to study brain plasticity and stimulus-evoked hyperexcitability (Goddard et al., 1969; McIntyre et al., 2002). By examining the effect of multiple METH treatment on kindling acquisition, the present study suggests that an acute METH challenge facilitates the development of a hyperexcitable network. Multiple METH treatment (4 x 10 mg/kg, s.c.) increased the single afterdischarge duration (ADD); however, the total ADD to reach a certain stage is not different between saline- and METH-treated groups. This finding suggests that the total ADD to reach a fully kindled state is constant. Thus, METH treatment did not facilitate amygdala kindling by lowering the total ADD, but lengthened each single ADD so that less stimulations were required to reach the limit for a certain seizure stage (Fig. 4.13). In summary, results obtained from this investigation suggest that METH exposure enhances the risk and rate of epileptogenesis in patients who experience seizures or those with abnormally lowered seizure threshold.

Kindling is undoubtedly regulated by some inhibitory systems (Corcoran and Mason, 1980). Numerous studies have demonstrated that monoamines play a significant inhibitory role in limiting the development of amygdala kindling (Arnold et al., 1973; Corcoran et al., 1974; Callaghan et al., 1979; McIntyre et al., 1979; Corcoran and Mason, 1980). Previous research has attempted to investigate which monoamine system(s) is/are involved in the kindling process using a pharmacological approach by selectively injecting or depleting certain monoamines. The results indicate that 5-HT and NE play a more prominent role as compared to DA in preventing amygdala kindling acquisition (Mohr and Corcoran, 1981; Albala et al., 1986; see Burchfiel et al., 1986; Lerner-Natoli, 1987; see Corcoran, 1988; Kirkby and Kokkinidis, 1991). Central depletion of 5-HT or NE, but not necessarily DA,
Figure 4.13. Diagram illustrating the proposed model for METH-induced facilitation of amygdala kindling. Numbers from 1 to 11 represent days of kindling and stimulations (one stimulation per day). Since the total afterdischarge duration (ADD) to reach a certain seizure stage is constant for both saline- and METH-treated groups, METH prolongs the single ADD so that fewer stimulations are required for METH-treated rats to reach stage 3 or stage 4/5 seizures compared with controls.
facilitates kindling development (Lerner-Natoli, 1987; Bortolotto and Cavalheiro, 1986). Therefore, in spite of being an important marker for METH-induced neurotoxicity (Fukumura et al., 1998), the striatal DA depletion observed in the present study (Fig. 4.8) is not likely to be involved in the facilitation of amygdala kindling.

Although the decreased NE activity facilitates kindling development (Bortolotto and Cavalheiro, 1986), the NE system was resistant to METH challenge and NE levels were unaltered as shown in Fig. 4.9. This finding argues against the role of NE in METH's ability to enhance kindling acquisition.

The present study demonstrates that 5-HT is depleted throughout the limbic system and striatum after multiple METH treatment (Fig. 4.7). Numerous reports have suggested that 5-HT plays an important inhibitory role in kindling (Lerner-Natoli, 1987). Stimulation of the 5-HT-rich median raphe blocks amygdala kindling (Siegel and Murphy, 1979). Conversely, destruction of serotonergic terminals in amygdala with the selective 5-HT neurotoxin 5, 6-dydroxytryptamine (5, 6-DHT) results in the facilitation of amygdala kindling (Lerner-Natoli, 1987). It is therefore inferred that METH-induced toxicity of 5-HT depletion is involved in mediating the facilitation of amygdala kindling. This hypothesis is supported by results in the present investigation where multiple METH treatment did not deplete 5-HT in younger rats (Fig. 4.12) nor did it facilitate amygdala kindling acquisition in this group (Fig. 4.11).

The role of 5-HT in suppressing kindling acquisition is further supported by the finding that the selective serotonin reuptake inhibitor (SSRI) fluoxetine suppresses focally evoked limbic motor seizures (Torta and Monaco, 2002). Furthermore, the anticonvulsant drugs lamotrigine and carbamazepine, which counteract the kindling acquisition (Stratton et al., 2003; Gilbert and Teskey, 2007), have been shown to
elevate the extracellular level of 5-HT (Southam et al., 1998; Yan et al., 1992; Dailey et al., 1998). In addition, the anticonvulsant effects of phenytoin and carbamazepine are enhanced by fluoxetine (Leander, 1992). All the evidence above supports the anticonvulsant property of 5-HT.

The inhibitory role of 5-HT in amygdala kindling is probably due to its suppressing effect on neuronal excitability. Studies demonstrate that the amygdala receives a dense serotonergic projection from the dorsal raphe nucleus (Stutzmann et al., 1998) and 5-HT suppresses excitatory synaptic transmission in the basolateral amygdala (Cheng et al., 1998) and hippocampal CA1 pyramidal cells (Sprouse and Aghajanian, 1988; Salgado and Alkadhi, 1995; Lu and Gean, 1998). This effect was most likely mediated by the 5-HT 1A receptor, which is coupled with an inhibitory G-protein (Cheng et al., 1998; Lu and Gean, 1998). Thus, 5-HT1A receptor activation elicits a membrane hyperpolarizing response as a result of increased potassium conductance of G-protein-coupled inwardly rectifying K channel (GIRK), which exerts an anticonvulsant activity in various experimental in vivo as well as in vitro seizure models such as hippocampal kindled seizures in cats, intrahippocampal kainic acid-induced seizures in freely moving rats, and picrotoxin-, bicuculline-, and kainic acid-induced seizure-like electrographic activity in rat hippocampal slice preparations (Wada et al., 1993; Salgado and Alkadhi, 1995; Lu and Gean, 1998). In summary, the METH-induced kindling facilitation observed in this study may be related to a disinhibition which contributes to the epileptiform activity from the stimulated site (amygdala) due to the attenuation of 5-HT activity. In addition, hippocampal hyperexcitability facilitates amygdala kindling in rats (Mimajafi-Zadeh and Pourgholami, 2002). Collectively, it is thought that METH-induced neurotoxicity and subsequent 5-HT depletion in the amygdala and hippocampus increase the excitability
of the limbic system, facilitate the generation and spread of epileptic seizure and thus enhance the epileptogenic process.

Interestingly, previous studies suggest that increasing doses of METH pretreatment causes tolerance and reduces neurotoxicity induced by a high-dose METH challenge administration (Schmidt et al., 1985; Gygi et al., 1996; Johnson-Davis et al., 2003 & 2004). Therefore, it is anticipated that those METH-tolerant rats would not exhibit an enhanced amygdala kindling rate. As such, the METH-tolerance phenomenon would be a potentially useful model to confirm the role of 5-HT system in METH's influence on kindling development.
References


Hippocampus 1:41-66.


Teskey GC, Radford KS, Seif I, Dyck RH (2004) MAO(A) knockout mice are more susceptible to seizures but show reduced epileptogenesis. Epilepsy Res 59:25-34.


CHAPTER 5

DISCUSSION

A sharp increase in the number of methamphetamine (METH)-related emergency room visits has been observed in recent years according to the Drug Abuse Warning Network (U.S. Department of Health and Human Services). METH-induced seizures are one of the most common symptoms (Lan et al., 1998) and can be fatal if they progress to status epilepticus (SE), which is often resistant to standard anticonvulsant drugs (Lowenstein and Alldredge, 1993). Thus, an understanding of the mechanisms underlying METH-related seizures can have a potentially important impact on their clinical management and preventing the subsequent brain damage associated with METH overdose.

METH can directly induce seizures in mice and rats (Hanson et al., 1999). Chronic METH treatment tends to lower the hippocampal-kindled seizure threshold (Minabe and Emori, 1992), while prenatal METH exposure alters seizure threshold in a model-specific manner. For example, adult prenatally METH-exposed male rats display a decreased threshold of clonic seizure, whereas the tonic-clonic seizure threshold is not altered by prenatal METH exposure (Slamberova, 2005). Therefore, METH's effect on seizure threshold seems to be inconsistent. This dissertation systemically evaluated the hypothesis that METH modifies seizure threshold and epileptogenesis through its effect on monoaminergic neurotransmission.
Effect of METH Treatment on Seizure Threshold

In Chapter 2, we explored the effect of METH on the threshold for tonic hindlimb extension, 6-Hz psychomotor seizures, pentylenetetrazol (PTZ)-induced clonus and kainic acid (KA)-induced complex partial seizures with secondary generalization. The four seizure models cover a broad range including electroconvulsant and chemoconvulsant, generalized and partial seizures, and thus help establish a profile of METH's effects on seizure susceptibility. The results demonstrate that METH does modulate seizure threshold. However, it could be proconvulsant, anticonvulsant or exerts no effect on seizure threshold depending on the model employed, i.e., METH was found to lower the threshold for 6-Hz psychomotor seizure and PTZ-induced clonus, while it elevated the tonic hindlimb extension threshold. Interestingly, METH did not change the incidence or latency of KA-induced seizures.

Different brain structures are activated in the four seizure models employed (Browning and Nelson, 1986; Browning et al., 1993). As discussed in Chapter 2, the data suggest that METH enhances local excitability in the forebrain but inhibits the brainstem structures which gates seizure generalization. Single unit recording (Boraud et al., 2002) and c-fos staining (Barton et al., 2001) would be useful to help confirm this hypothesis.

Our finding that METH exerts opposing effects on tonic hindlimb extension and 6-Hz psychomotor seizures supports the theory that these seizures are mediated by separate networks, i.e., brainstem and forebrain, respectively (Browning and Nelson, 1986; Browning et al., 1993). Likewise, some anticonvulsant drugs have differential influences on seizure threshold in these models. For example, phenytoin is effective in the maximal electroshock (MES) test but not the 6-Hz model, whereas levetiracetam
METH seems to have no effect on the threshold for KA-induced seizures in the present study. This negative finding is of interest because it suggests that METH may not interact with the neuroanatomical pathway activated by KA. However, a lack of effect of METH in this model may be due to a large variability observed in the latency, duration and incidence of KA-induced seizures.

In summary, METH modulates seizure threshold in a model-specific manner. Subsequent studies attempted to delineate the neurochemical mechanisms underlying its effects on electroconvulsant seizure threshold.

**Dopamine Receptor Modulation Is Involved in METH's Modulation of Electroconvulsant Seizure Threshold**

Although there is considerable evidence that supports an important role of dopaminergic neurotransmission in modulating seizure threshold (see Starr, 1996), it is not clear whether dopamine (DA) is related to METH's effect on electroconvulsant seizure threshold. Results described in Chapter 3 indicate that the selective D-1 antagonist SCH-23390 (Iorio et al., 1983; Sandoval et al., 2002) and D-2 antagonist eticlopride (Hall et al., 1985; Prinssen et al., 2004) exerted synergistic and antagonistic effects with METH, respectively. In addition, eticlopride attenuated the SCH-23390/METH synergy in a dose-dependent manner. These data suggest that D-1 and D-2 receptors are playing contrary roles in mediating METH's effect on electroconvulsant seizure threshold.

The anatomical sites for D-1 and D-2 actions involved in seizure threshold control seem to be different: activation of the D-1 pathway in the substantia nigra pars reticulata (SNr) decreases the threshold for pilocarpine-induced seizures, while activation of the striatal D-2 receptor pathway leads to raised seizure threshold.
The SNr is a critical site involved in the propagation of generalized convulsions (Iadarola and Gale, 1982; Waszczak et al., 1986; Garant and Gale, 1987; Veliskova et al., 2002; Veliskova and Moshe, 2006), which contains mainly GABAergic neurons (MacLeod et al., 1980; Chevalier et al., 1985). The striatum is connected to SNr by direct (striato-nigral) and indirect (striato-pallido-subthalamic-nigral) pathways (for reviews, see Albin et al., 1989; Alexander and Crutcher, 1990; Bolam et al., 2000). The direct pathway is modulated by D-1 receptors and exerts GABA-mediated inhibition on SNr neurons, while the indirect pathway modulated by D-2 receptors sends excitatory glutamatergic projections to the SNr from the subthalamic nucleus (STN) (Gerfen et al., 1990, also see Surmeier et al., 2007). In addition, there is abundant expression of D-1 receptors in SNr, which are mostly located on the terminals of GABAergic striatonigral neurons (Porceddu et al., 1986; Trevitt et al., 2004).

Results obtained in this dissertation suggest that mechanisms underlying METH's effect on electroconvulsant seizure threshold are related to METH-induced release of dopamine and subsequent dopaminergic receptor activation in the brain. The D-1 receptor activation in the SNr results in an excitation of SNr by attenuating GABA-mediated striato-nigral inhibition ('direct pathway' of basal ganglia) (Matthews and German, 1986), whereas an activation of striatal D-2 receptor inhibits the SNr through the "indirect pathway" of the basal ganglia (see Albin et al., 1989; Alexander and Crutcher, 1990; Bolam et al., 2000; see Surmeier et al., 2007). It is thought that the D-2 action outweighs D-1 activity and thus leads to the net effect of decreased SNr activity under METH challenge. As the result, the target structures of SNr such as superior colliculus, pedunculopontine tegmental nucleus and thalamus are disinhibited, which causes an increase of the threshold for generalized tonic
extension seizures (Dean and Gale, 1989; Depaulis et al., 1990; Redgrave et al., 1992; Shehab et al., 2005) and a decrease of limbic motor seizure threshold (Cassidy and Gale, 1998). It is noted that the D-1 receptors in the striatum seem not to be involved in seizure control (Turski et al., 1990). In summary, METH's effect on electroconvulsant seizure threshold is mediated by modulating dopaminergic neurotransmission, i.e., the D-1 receptors in SNr and D-2 receptors in striatum exert opposite effects on the SNr excitability and thus seizure susceptibility.

These findings are supported by previous studies demonstrating that the anticonvulsant action of dopamine was attributed to the D-2 receptor stimulation in forebrain (Loscher and Czuczwar, 1986; Turski et al., 1990; see Starr, 1996), while the D-1 receptor activation in midbrain exhibits proconvulsant effect (Turski et al., 1990; see Starr, 1996).

Based on the discussion above, the D-2 agonist quinpirole (Kebabian et al., 1997; Truong et al., 2004) was hypothesized to elevate the threshold for tonic hindlimb extension and decrease the 6-Hz psychomotor seizure threshold. However, we observed a proconvulsant effect of quinpirole (20 mg/kg, i.p.) in both models. The discrepancy might be due to the possibility that quinpirole, at the high dose of 20 mg/kg, may have lost its selectivity to D-2 receptors and activate other systems such as norepinephrine (NE) (Fuller and Hemrick-Luecke, 2007), which modulate the seizure threshold as well (see Giorgi et al., 2004).

**METH Enhances Amygdala Kindling Acquisition**

The results described in Chapter 4 indicate that multiple METH treatment facilitates amygdala kindling acquisition by reducing the number of stimulations required to reach stage 3 or stage 4/5. It suggests that METH exposure might enhance the risk or facilitate the development of epileptogenesis in those individuals with
epilepsy or predisposed to develop epilepsy.

The afterdischarge duration (ADD) of amygdala kindling was prolonged by METH treatment in rats. However, the afterdischarge threshold (ADT), i.e., the minimal current intensity that can induce an afterdischarge lasting more than 3 sees, was not different for saline- vs. METH-treated animals, nor was total ADD to reach a certain seizure stage. The data suggest that the neurotoxicity associated with multiple METH treatment does not lower the threshold for a focal seizure but it does appear to lengthen an electrographic seizure once it is triggered. Thus, it is speculated that some inhibitory mechanism(s) in the kindled rats is/are compromised. The monoamines norepinephrine (NE) and serotonin (5-HT) have both been demonstrated to possess the ability to counteract kindling acquisition (McIntyre, 1981; Bortolotto and Cavalheiro, 1986; Lerner-Natoli, 1987). The effect of METH on brain monoamine levels was assessed in an effort to determine whether changes in one or more of the monoamines could contribute to the facilitated kindling acquisition observed in METH-treated rats.

**Multiple METH Treatment-induced Monoamine Neurotoxicity**

Multiple METH treatment (4 x 10 mg/kg, s.c.) caused hyperthermia and the long-term neurotoxicity characterized by monoamine depletion. For example, METH treatment reduced 5-HT concentrations in the limbic brain structures (amygdala and hippocampus) and striatum. However, the NE system was relatively resistant to METH challenge and its level remained unaltered. A large body of evidence supports the inhibitory role of 5-HT in kindling development (Lerner-Natoli, 1987). For example, amygdala kindling can be blocked by increasing 5-HT release from raphe nucleus (Siegel and Murphy, 1979) and facilitated by 5, 6-dihydroxytryptamine (5, 6-DHT)-induced 5-HT depletion in the amygdala (Lerner-Natoli, 1987); the selective...
5-HT reuptake inhibitor (SSRI) fluoxetine suppresses focally evoked limbic motor seizures (Prendiville and Gale, 1993). Therefore, the data suggest that METH might enhance amygdala kindling by depleting 5-HT in the brain. This hypothesis is supported by our observation that METH treatment did not facilitate kindling acquisition in younger rats that did not display monoamine depletion in response to METH treatment.

The inhibitory effect of 5-HT in the kindling model is thought to be mediated by the 5-HT 1A. Previous research has suggested that 5-HT 1A receptor activation elicits a membrane hyperpolarizing response as a result of increased potassium conductance of G-protein-coupled inwardly rectifying K\textsuperscript{+} channel (GIRK). This action has an anticonvulsant effect in various experimental seizure models (Wada et al, 1993; Salgado and Alkadhi, 1995; Lu and Gean, 1998).

Likewise, galanin, a 29-amino acid neuroendocrine peptide, is a powerful inhibitor of seizure activity and hippocampal kindling (see Mazarati, 2004). The anticonvulsant effect of galanin is thought to be mediated by both galanin type 1 (GalR1) and type 2 (GalR2) receptors (Mazarati et al., 2006). The coupling of GalR1 to Gi protein opens G-protein-coupled inwardly rectifying K\textsuperscript{+} channels (GIRK) or ATP-sensitive K\textsuperscript{+} channels, which causes the presynaptic inhibition of glutamatergic neurons (Mazarati et al., 2006; see Lundstrom et al., 2005). The depletion of galanin or galanin receptors through genetic mutations or antisense technique results in a lowering of seizure threshold (Mazarati et al., 2000, 2004a & b; Jacoby et al., 2002).

Although multiple METH treatment depletes striatal DA, the literature suggests that DA depletion does not affect kindling development (McIntyre, 1980; Kirkby and Kokkinidis, 1991), and thus DA is not thought to contribute to the facilitation of kindling induced by METH treatment.
In addition, METH induces a broad range of changes at DA and 5-HT receptors (McCabe et al., 1987; Doudet and Holden, 2003), in glutamatergic neurotransmission (Zhang et al., 2001), GABA-mediated inhibition (Mark et al., 2004), neuropeptide Y (Horner et al., 2006) and neurogenesis (Powrozek et al., 2004), all of which might have direct or indirect impact on neuronal excitability and kindling. More research is needed to determine whether other factors contribute to METH's ability to enhance epileptogenesis.

**Overall Conclusions**

The present study provides experimental evidence demonstrating that METH acutely modulates the seizure threshold in a model-specific manner and has a long-term effect of enhancing epileptogenesis. In addition, dopaminergic neurotransmission is involved in METH-induced change of electroconvulsant seizure threshold but does not appear to contribute to facilitated epileptogenesis. The neurotoxicity of multiple METH treatment, particularly 5-HT depletion, is thought to mediate its facilitation of amygdala kindling. In summary, METH modifies seizure threshold and epileptogenesis through selective effects on monoaminergic neurotransmission.

**Future Directions**

The present study defined the behavioral effect of METH on seizure threshold and epileptogenesis and investigated the monoamine mechanisms involved. In an effort to further delineate the neurochemical and neuroanatomical mechanisms of METH to modify seizure/epilepsy, additional studies are clearly warranted. The data obtained so far support the following future studies: 1) conduct single unit recording to confirm the neuroanatomical pathway mediating METH's effect on seizure
threshold; 2) assess the role of other neurotransmitters/neuropeptides such as
neuropeptide Y, glutamate, GABA in mediating METH's effect on seizure threshold
and epileptogenesis; 3) evaluate the anticonvulsant pharmacology of METH-related
seizures. The studies above would help further our understanding of the relationship
of METH to seizure/epilepsy and hopefully provide important information for the
clinical treatment of METH-related convulsions and subsequent pathology.
References


Fuller RW, Hemrick-Luecke SK (1985) Decrease in hypothalamic epinephrine concentration and other neurochemical changes produced by quinpirole, a


Horner KA, Westwood SC, Hanson GR, Keefe KA (2006) Multiple high doses of methamphetamine increase the number of preproenkephalin mRNA-expressing neurons in the striatum of rat via a dopamine D1 receptor-dependent mechanism. J Pharmacol Exp Ther 319:414-421.


Loscher W, Czuczwar SJ (1986) Studies on the involvement of dopamine D1 and D2...


