Chapter V

Discussion and Conclusion

A diversity of animal models are available for the study of epilepsy and these models have a proven history in advancing our understanding of basic mechanisms underlying epileptogenesis and have been instrumental in the screening of novel antiepileptic drugs (Matthew and Sarkisian, 2001). Convulsive seizures, characterized by tonic-hindlimb extension are reliably induced by maximal electroshock which continues to be a popular method whereas pentylenetetrazole (PTZ) is probably the most widely used systemically administered convulsant. PTZ can be used to elicit absence-like seizure. (Matthew and Sarkisian, 2001)

The maximal electroshock (MES) and the Pentylenetetrazole (PTZ) seizure tests were used in this experiment for screening anticonvulsant activity of Phthalimide derivative, AMP, compared with conventional antiepileptic drugs, VPA (Rogawski and Porter, 1990).

The fact that phthalimide is a potent anticonvulsant activity, however, with high toxicity, attracted many investigators to further modify its structure searching for phthalimide derivative with improving pharmacological profiles (Clark et al, 1984). AMP is one among them. AMP was more effective than VPA in MES and as approximately effective as VPA in PTZ test. The median effective dose (ED_{50}) of AMP were 17 and 68 mg/kg B.W. in MES and PTZ test respectively while corresponding values for VPA were

214 and 86 mg/kg B.W.(Table 4). Peak effect of AMP and VPA were observed at 30 min after intraperitoneal administration.

Regarding to duration of anticonvulsant activity, it is apparent that intraperitoneal administration of either AMP or VPA exhibited protection against MES at least until 3 hours after dosing, however, with an increment of the ED_{50} values (Figure 5) whereas, no any effects were seen at 6 hours (results not shown). As illustrated in Figure 5, the ED_{50} of AMP were always lower than those of VPA at any given time. This finding confirms the higher potency of AMP than VPA throughout the observation period.

Most antiepileptic drugs suffer from a broad range of undesirable side effects such as sedation, cognitive disorder, impairment of motor function and other adverse effects (Rall and Schlcifer, 1990). Rotorod test of Dunham and Miya (1957) is the most commonly used screening test to estimate the minimal neurological deficit in experimental animals (Loscher, Nolting and Fassbender, 1990). As shown in Figure 8, AMP in the dose approximately 2 times higher than those needed to protect against MES, inhibited rotorod performance. Neuroprotective indices (PI = TD_{50} /ED_{50}) were found to be 2.24 for AMP and 1.44 for VPA (Table 5). As it has been previously proposed that compound with an estimated PI of at least 2 in MES model should proceed to further evaluation (Losher and Nolting, 1991), the present finding substantiates such opportunity for AMP. For VPA, it was found that the PI is not only well within the range of conventional antiepileptic drugs (Stagnitto et al., 1990; Loscher and Nolting, 1991) but also in good

agreement with those previously reported from this laboratory (Ponchulee Supatchipisit, 1995) and other investigators (Loscher and Nolting, 1991).

With regards to the effect on barbiturate sleeping time, no statistical significance was noted between effects of NSS and PEG 400 (Figure 10). The barbiturate sleeping time was significantly prolonged by both low and high dose of AMP (17 and 70 mg/kg B.W.) whereas for VPA, no significant effect prolongation of the barbiturate sleeping time was demonstrated by either low or high dose of VPA. The finding suggests a lower degree of CNS depression of VPA than AMP.

As shown in Table 5, for acute toxicity, VPA is lesser toxic than AMP (LD_{50} = 605 vs 101 mg/kg). However in term of the relative safety margin, indicating discrepancy between the effective anticonvulsive dose and lethality, the relative safety margin of AMP was higher than that of VPA in the MES test whereas opposite results was observed in the PTZ test (5.94 vs 2.83 in MES test and 1.49 vs 7.03 in PTZ test).

Similarity in anticonvulsant profile exhibited by AMP and VPA, it could suggest that AMP may become a broad spectrum antiepileptic drug as VPA which mostly being used in the treatment of absence seizures as well as generalized tonic-clonic and partial seizures. However as illustrated in Table 5, the ED_{50} of AMP in PTZ test is lower than its TD_{50} suggesting high degree of motor impairment in the dose that can prevent seizure. In line with the finding, depressant effect of AMP was also evident in prolongation of

barbiturate sleeping time. Together with the result that the LD_{50} of AMP was about 1.5 times of its effective dose, it is likely for AMP to be further developed for seizure type correlate well with MES but not as a broad spectrums AED such as VPA.

An imbalance between excitation and inhibition has been associated with the generation of epileptic pathological condition, both in animal models and in humans. From mechanistic point of view, potentiation of inhibitory neurotransmitters namely, GABA and glycine, and/or diminution of excitatory neurotransmitters such as glutamate and aspartate have become potential targets of new AEDs (Upton, 1994; Schwartzkroin, 1997).

Based on our finding that the ED_{50} against PTZ-induced convulsion of AMP was 68 (53-87) mg/kg B.W. the dose of 70 and 100 mg/kg B.W. were selected for further investigations.

Due to hydrophobic nature of the tested compounds, PEG400 was used to solubilize them. Apparently, as shown in Figures 11, 12, 13 and 14, no statistically difference on the level of aspartate, glutamate, glycine and GABA were observed between NSS- and PEG400 – treated groups Thus, it is justified to use PEG400-treated group as a sole control group for further comparison of AMP-and VPA-treated groups.

In previous studies, VPA has been shown to increase GABA concentrations in whole brain (Godin et al., 1969) and synaptosomes

(Loscher, 1981; Loscher and Vetter, 1985). VPA appeared to preferentially enhance GABA turnover in neuronal compartment (Ladarola and Gale, 1981) and this might be expected to increase GABAergic transmission. Biggs, Peace and Whitton (1992) had performed experiments on ventral hippocampus of freely moving rats and found that sodium valproate (400 mg/kg B.W.) had increased GABA level. However, contradictory results of VPA, though in line with the finding of Yeamvanichnum (1997), Numthongsakun (2000) and Wanasuntronwong (2001), was observed in the present study, VPA in the dose of either 200 and 400 mg/kg B.W. had no effect on brain GABA level (Figure 18 and 22). This discrepancy might resulted from different area of the brain was used in the present study. In addition, level of neither glycine nor glutamate was found to be affected by VPA albeit a significant reduction in the level of aspartate was seen at 140 and 160 min.

For glutamate, it was found that VPA 400 but not 200 mg/kg B.W. significantly decreased the level of brain glutamate (Figures 16 and 20). Similar result was reported from this laboratory by Numthongsakun (2000) and Wanasuntronwong (2001).

In comparison to VPA, AMP exhibited different profile on brain amino acid levels. AMP (70 mg/kg B.W.) significantly decrease the level of glutamate (Figure 20) and increase the level of glycine and GABA (Figure 21 and 22) whereas slightly but not significant decrease was noted on the aspartate level (Figure 19).

Glycine plays a role as an inhibitory neurotransmitter in CNS. Its receptor is a ligand-gated Cl^- channel which was found at pyramidal neurons in cerebral cortex (Nass, et al., 1991; Becker, Betz and Schroder, 1993). Glycine receptor promotes the Cl^- influx thus provoking hyperpolarization with a decrease in cellular excitation state is which are known to suppress seizures (Olsen and Delorey, 1999). Based on the finding in the present study that AMP 70 mg/kg B.W. significantly increased the level of glycine (Figure 21), it was suggested that an increment of the glycine level might be responsible for the anticonvulsant action of AMP.

With regards to the level of GABA, it is the predominant inhibitory neurotransmitter in the mammalian CNS (Olsen & Avoli, 1997). Impairment of GABA function is widely recognized to provoke seizures, whereas facilitation has an anticonvulsant effect (Loscher, 1999). Several AEDs exert their effects, at least in part, by actions on the GABAergic system. Increased GABA synthesis, increased release, allosteric receptor facilitation, and reduced inactivation have all been implicated in the mechanisms of action of commonly used agents (Sills, Butler and Thompson, 1999) eg. phenobarbital, benzodiazepine, vigabatrin and tiagabine. The GABA system also represents the most successful target for the rational design of novel antiepileptic compounds (Loscher, 1998) as it appears to be for AMP (70 mg/kg B.W.).

Glutamate play an important role in the initiation and spread seizure activity via excitatory action on ligand- gated ion channels (NMDA and non-NMDA receptors) to increase the influx of sodium and calcium ions whereas

its effect on metabotropic glutamate receptor is unlikely to play a significant role in seizure or epileptogenesis (Chapman, 1998). In contrast to the AMPA and the kainate receptor which are the non-NMDA receptors, activation of the NMDA receptors by glutamate requires glycine as co-agonist. Thus increasing amount of glycine may attenuate response of the receptor to NMDA. In addition to postsynaptic modulation of NMDA receptor by glycine previously described. Presynaptic modulation to reduce glutamate release from glutamatergic neurone by agents acting primarily on voltage-gated sodium channels has been proposed to account for anticonvulsant activity of some AEDs, eg. lamotrigine, riluzole and BW-619c89 which were often referred to as glutamate release blockers (Taylor and Meldrum, 1995).

Microdialysis used in the present study clearly demonstrated a decreased level of glutamate by AMP 70 mg/kg B.W. Therefore a reduction of excitation neurotransmitter as well as an increase in inhibition neurotransmitter namely GABA and glycine may act in concert to account for anticonvulsant activity of AMP 70 mg/kg B.W. However these were not the case for AMP 100 mg/kg B.W. which did not cause any alteration in the level of either excitatory or inhibitory neurotransamitter. Taken into consider action that the LD_{50} of AMP in mice was only 2 times higher than its ED_{50} in PTZ test, it is likely that AMP 100 mg/kg B.W. might be toxic dose that could trigger some other mechanisms to counterbalance the effects observed in low dose (70 mg/kg B.W.) Similarly, biphasic effect of CBZ on dopamine system has been reported by Okada et al, (1997) and different response of

brain monoamine level was exhibited by non-toxic and toxic concentration of CBZ.

In conclusion, the present study demonstrated anticonvulsant activity of AMP against both the MES and PTZ tests. However due to unfavorable neuroprotective indices ($TD_{50}/ED_{50} = 0.56$) and low relative safety margin ($LD_{50}/ED_{50} = 1.49$) of AMP in PTZ model, AMP is anticipated to be clinically beneficial for certain types of seizure. Reduction of glutamate in concert with increment of GABA and glycine seem to underlie anticonvulsant observed in vivo.

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