Chapter IV

Discussion and conclusion

The MES and PTZ models represent the highly reproducible *in vivo* systems which are the most commonly used for antiepileptic drugs (AEDs) screening models. Results in these two models are employed in the search for effective anticonvulsant drugs and sometimes a test of possible mechanisms of action as well. The MES test is an excellent animal model for the identification of new AEDs that block seizures spread and are likely to be effective for the management of generalized tonic-clonic seizure in human. On the other hand, the PTZ test is an effective model that identifies the AEDs that raise seizure threshold and are likely to couple with AEDs that are effective in the treatment of absence seizure (Rogawski and Porter, 1990; White, 1997; Löscher ,1998). In addition, the PTZ- induced clonic seizure are blocked by drugs acting at the $GABA_A$ receptor e.g. benzodiazepine and phenobarbital whereas the hind limb extension observed in MES test is effectively blocked by AEDs such as phenytoin and carbamazepine which are known to inhibit voltage-sensitive sodium channels (Rogawski and Porter, 1990; White, 1997).

In accordance with previous work, the present studies demonstrated the anticonvulsant activity of VPA in protection against seizure in both MES and PTZ models (Ferrendelli, Holland, and Covey,1989; Pornchulee Supatchaipisit, 1995; Thongchai Sooksawate, 1995) whereas VPM exclusively exerted its anticonvulsant activity in the MES (ED_{50}=107 mg/kg B.W. at pretreated time of 60 min) but not the PTZ model. Taking into consideration that lamotrigine, the new AED which exerts it anticonvulsant activity by selective blockade of sodium channels of glutamatergic neurones, posses the same profile of anticonvulsant activity in these two models similar to those exhibited by phenytoin and carbamazepine (White, 1997; Rho and Sankar, 1999). It is suggestive that VPM may exert its anticonvulsant activity in the same manner as did its precedents.

Apparently, VPM was more effective than VPA which exhibited its ED_{50} of 230 mg/kg B.W. at pretreated time of 30 min in MES test. Furthermore, despite rather similar

profile of lethality, VPM seems to posses higher margin of safety than that of VPA (5.89 vs 2.97, Table 2) implying that VPM may clinically more potent and safer than VPA.

Most AEDs suffer from unwanted effect such as ataxia, sedative and impairment of motor function (Deckers et al., 1997). Rotorod test of Dunham and Miya (1957) is the most commonly used screening test to estimate the neurological deficit in experimental animals which show the motor impairment such as muscle incoordination or relaxation (Löscher, Nolting and Fassbender, 1990). Mice receiving either NSS or PEG400 were able to maintain their equilibrium on the rotating rod. However, neurological deficit in a dose dependent manner was noted in experimental animal being intraperitoneally injected by either VPM or VPA. As shown in Table2, the median neurotoxic dose (TD_{50}) of VPM was 151 mg/kg B.W. resulting in protective index ($PI=TD_{50}/ED_{50}$) of 1.41 and 1.34 for VPM and VPA, respectively. Therefore, it can be anticipated that therapeutic dose of VPM should be able to produce neurological deficit in terms of motor impairment to the same extent as does the therapeutic dose of VPA.

Since no statistical significance was observed among the effect of VPM, VPA and PEG400 (Figure12 and 13), it is suggestive that VPM (100 and 200 mg/kg B.W.) and VPA had no significant depressant effect on locomotor activity. The depressant effect deviated from those of NSS could be presumably accounted by the solvent used, PEG400. Failure of VPA (100 and 250 mg/kg B.W.) to depress locomotor activity corresponds well with previous work reporting that VPA in the dose range of 10-400 mg/kg B.W. i.p. had no significant effect on locomotor activity and no evidence of behavioral effect was noted until the dose of 500-600 mg/kg B.W. of VPA was reached (Anlezark et al, 1976; File and Aranko, 1988).

Furthermore, based on the result that the hypnotic dose of VPM was only about 2 times higher than its ED_{50} against MES, VPM may comparatively be more sedative than VPA. This may explain also the observation that VPM significantly prolong barbiturate sleeping time more pronounced than those exhibited by VPA (Figure14). However, in addition to such pharmacodynamic interaction between VPM and pentobarbital, pharmacokinetic interaction between these two compounds might play role in prolongation of barbiturate sleeping time as well. Interaction by inhibiting metabolizing

enzyme of concurrently administered antiepileptic drug is clearly exemplified by the co-administration of phenytoin and phenobarbital (Anderson, 1998). To clarify this possibility, the effect of VPM on cytochrome P450, the metabolizing enzyme of pentobarbital should be further investigated.

In general, imbalance between excitatory and inhibitory amino acid neurotransmission resulting in hyperactivity of the brain may account for epilepsy. Thus, 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)

In the present studies, VPA in the dose of 250 mg/kg.B.W. significantly decreased the total amount of glutamate but not aspartate(Figure18 and 17) whereas no significant change in the levels of glycine and GABA was observed (Figure19 and 20). Different profile of effects on the amount of amino acid neurotransmitters was demonstrated by VPM. As shown in Figure18 VPM 200 but not 100 mg/kg B.W. significantly decreased the amount of glutamate whereas the level of aspartate, though tended to be reduced was not significantly affected by the administration of VPM (Figure17). Furthermore, VPM 100 but not 200 mg/kg. B.W. significantly decreased the amount of glycine(Figure19) whereas the level of GABA in VPM-treated group did not significantly deviate from those receiving PEG400(Figure20). Apparently, in terms of effect on cortical amino acid neurotransmitters, VPM behaved rather differently from its parent compound, VPA.

Glutamate plays a key role in the initiation and spread of 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 seizures or epileptogenesis (Chapman, 1998). In contrast to the AMPA and the kainate receptors, which are the non-NMDA receptors, activation of the NMDA receptors by glutamate requires glycine as co-agonist. Measurement of glycine in cerebrospinal fluid indicates that it is present in sufficient amount in the vicinity of the NMDA receptor to enable activation by an

NMDA-recognition site agonist (Chapman, 1998). Low concentration of glycine attenuate response of the receptor to NMDA (Kemp and Lesson, 1993). Therefore, in opposition to the inhibitory effect of glycine exerted on the strychnine-sensitive glycine receptors in the spinal cords, interaction of glycine to strychnine-insensitive binding site on cortical NMDA receptors results in positive effect of NMDA-recognition site agonist (Chapman, 1998). Thus, decrement of cortical glycine as noted in VPM-treated group may in part account for its anticonvulsant effect.

In addition to postsynaptic modulation of NMDA receptors by glycine previously described, presynaptic modulation to reduce glutamate release from glutamatergic neurones by agents acting primarily on voltage-gated sodium channels has been proposed to account for anticonvulsant activity of lamotrigine, riluzole and BW-619C89 which were often referred to as glutamate release blockers (Taylor and Meldrum, 1995). Taking into account that VPM significantly decreased the level of cortical glutamate and that VPM exhibited anticonvulsant profile in MES but rather ineffective in PTZ tests in the same fashion as those of phenytoin, carbamazepine and lamotrigine, it can be speculated that like lamotrigine, VPM may exert its anticonvulsant effect on glutamate release via blockade of the sodium channels.

In conclusion, the present studies demonstrated a potent and rather selective anticonvulsant activity of a new valproic analog, VPM. In contrast to its parent compound, VPM was ineffective in PTZ model, rather sedative and seemed to possess different mode of anticonvulsant activity from those of VPA as reflected by ability of VPM to reduce cortical glycine and glutamate whereas VPA reduced only the levels of cortical glutamate. VPM seemed to be safer than VPA in therapeutic dose. However, VPM and VPA were probably able to produce rather similar degree of motor impairment. Further modification of the compound may lead to a better anticonvulsant valproic analogue or valproic analogue with a hypnotic activity.