Chapter IV

Discussion and conclusion

Imbalance between excitatory and inhibitory amino acid neurotransmitters resulting in hyperactivity of the brain may account for epilepsy (Rogawski and Porter, 1990; Upton, 1994). 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). Thus VHA, which is a novel derivative of valpromide, the primary amide of valproic acid was synthesized in an attempt to obtain a new compound with higher potency and less toxicity than VPA. As VHA was previously reported of being a potential anticonvulsant candidate in mice (Thongsathean, 1999), it was investigated for effects on the level of brain amino acid of freely moving rats in the present study. Based on our finding that the ED_{50} of VHA was 80(51-124) mg/kg B.W. in rats the dose of 100 and 200 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 Figure 7, 8, 9 and 10, 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 VHA- 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). Significantly, VPA appears to preferentially enhance GABA turnover in neuronal compartment (Ladarola and Gale, 1981) and this might be expected to increase GABAergic transmission. Although Farrent and Webstar (1989) failed to observe any change in spontaneous nigral GABA release by VPA, Silverman et al. (1991) reported that VPA enhanced cerebral GABA synthesis. Furthermore Biggs et al. (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 Numthongsakun (2000) and Yeamvanichanun (1997), was observed in the present study, VPA in the doses of either 200 and 400 mg/kg B.W. had no effect on brain GABA level (Fig 14 and 18). With regard to glycine which is an inhibitory neurotransmitter at strychnine-sensitive glycine receptor but a co-agonist of glutamate on NMDA receptor (Cooper, Bloom and Roth, 1996), it was found that VPA (200 and 400 mg/kg B.W.) had no effect on brain glycine level (Fig 13 and 17) and this finding was in agreement with the results of Numthongsakun (2000).

The decrease of an excitatory amino acid could participate in the anticonvulsive effect of VPA (Johannesson, 2000). VPA decreased aspartate levels in whole brain of mouse and rat after treatment with 200 and 400 mg/kg B.W. (Loscher and Horstermann, 1994). However, different results were observed by microdialysis method used in the present study. VPA had no effect on aspartate levels (Fig 11 and 15) and these finding were previously reported by Bigg et al. (1992) in freely moving rats. For glutamate which is the major excitatory amino acid neurotransmitter in the brain (Chapman, 1998: Dodd et al., 2000; Shank, Smith-Swintosky and Twyman, 2000), it was found that VPA 400 but not 200 mg/kg B.W. significantly decreased the level of brain glutamate (Fig 12 and 16). Similar result was reported from this laboratory by Numthongsakun (2000).

In comparison to VPA, VHA exhibited different profile on brain amino acid level. We found that VHA 100 mg/kg B.W. had no effect on the levels of aspartate, glutamate and GABA but it significantly increased the level of glycine (Fig 13 and 17). In high dose (200 mg/kg B.W.), VHA significantly increased the levels of both glycine and GABA (Fig 13, 14, 17 and 18). 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 (Naas, et al., 1991; Becker, Betz and Schroder, 1993). Based on the finding in the present study that VHA 100 and 200 mg/kg B.W. significantly increased the level of glycine (Fig 13 and 17), it was suggested that induction of the glycine level might be responsible for

the anticonvulsant action of VHA. 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). With regards to the result that only VHA in the dose of 200, but not 100 mg/kg B.W., significantly increased the level of GABA, it was unlikely that the increment of GABA was the primary mechanism underlying anticonvulsant effect observed in rats. However, the increment of GABA could definitely be a part of anticonvulsant effect of VHA in high dose. In parallel with increment of glycine and GABA levels other mechanisms such as blockage of Na^+ channel, post synaptic modulation of excitatory and/or inhibitory neurotransmitter or some other mechanisms (Loscher, 1998) should be further investigated in search for other possible mechanisms accounted for the anticonvulsant activity of VHA observed in rats.

In conclusion, the present study demonstrated the effect of VHA on extracellular amino acid levels such as aspartate, glutamate, glycine and GABA on freely moving rats. These results showed that VHA significantly increased glycine level in the dose of 100 and 200 mg/kg B.W. and significantly increased GABA level only in high dose but not at the dose closed to its ED_{50}. Therefore, it was suggested that at least, the increase in the level of inhibitory neurotransmitter, glycine, could account for the anticonvulsant activity of VHA in the dose of 100 mg/kg B.W. However in high dose (200 mg/kg B.W.), in addition to an increase in glycine, the increment of GABA should participate in anticonvulsant effect of VHA. Some other mechanisms than these two mechanisms should also be further elucidated.