Chapter IV

Discussion

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).

HPP, a novel amide derivative of VPA, was synthesized in an attempt to obtain a new compound with higher potency in anticonvulsant effect than VPA. The results of Supatchaipisit (1995) demonstrated that HPP was a relative potent, rapidly acting and orally effective anticonvulsant in maximal electroshock seizure (MES) and the pentylenetetrazole (PTZ) tests. However, HPP was rather ineffective in convulsive models induced by an intraperitoneal injection of $GABA_A$ receptor antagonist (bicucullin) or glycine receptor antagonist (strychnine). Therefore it is suggestive that the anticonvulsant activity of HPP observed in previous study is not directly related to $GABA_A$ or glycine receptors. Based on the result that HPP was highly active against PTZ which is generally known to diminish GABA's inhibition indirectly, some GABA-related effect other than direct effect at $GABA_A$ receptor site or some of excitatory amino acid neurotransmitter effect could be involved.

In an attempt to investigate effects described above, this study was focused on the effect of HPP, in comparison to VPA, on the level of excitatory and inhibitory amino acid neurotransmitters in cerebral cortex of freely moving rats by using microdialysis technique. It was found that HPP (80 and 160 mg/kg B.W.) had no effect on the cortical levels of inhibitory neurotransmitters GABA and glycine, Contradictory results with the finding of Supatchaipisit (1995) who found that HPP in high dose significantly increased cortical GABA level in rats. Negative result of HPP on the cortical level of GABA in this study might be due to in the difference of experimental models.

Previous study measured cortical GABA level in anesthetized rats whereas this study freely moving rats were used. In contrast to previous report by Supatchaipisit (1995), VPA (220 and 440 mg/kg) did not significantly change the cortical level of inhibitory neurotransmitters, GABA. However, the lack of VPA effect on the level of GABA measured in this study was in line with the finding of Numthongsakun (2000) and Yeamvanichanun (1997). Accordingly, inconsistent results of VPA also exist between various laboratories. VPA was reported to increase whole brain GABA levels in rodent after acute administration of VPA (Hariton et al., 1984). More interestingly, regional changes in GABA levels have been found in striatum, hippocampus, and cerebellum in rats after VPA treatment (Chapman et al., 1982), whereas Farrent and Webstar (1989) failed to observe any change in spontaneous GABA release by VPA in substantia nigra. It should be noted that different region of the brain were used to assess the effect of VPA, this may account for the difference observed. However, it is generally accepted that more than one mechanism is responsible for antiepileptic activity of VPA. With regard to glycine, it was found that VPA in this study exhibited no effect on cortical glycine level and this finding was in agreement with the results of Numthongsakun (2000) and Wanasuntronwong (2001). Glycine, which is a neurotransmitter in the spinal cord was not altered by VPA (Godin et al., 1969) but some reports found that concentrations of glycine increased in brain tissue (Löscher and Horsetermann, 1994). However, there is no evidence that the effects on glycine are relevant to the anticonvulsant effect of VPA.

Interestingly, the results of the present studies demonstrated that HPP could significantly decreased cortical excitatory glutamate level in freely moving rats, although the other excitatory amino acid neurotransmitter, aspartate did not significantly altered by HPP. With regards to VPA, only high dose of VPA did significantly decreased glutamate level, whereas it did not decreased cortical aspartate level. Similar result was reported from this laboratory by Wanasuntronwong (2001). The decrease in extracellular glutamate levels seen in this experiment demonstrated the pre-synaptic effect of HPP on glutamatergic system. It is likely that a reduction of cortical glutamate is the principal mechanism responsible for anticonvulsant activity of HPP. The inhibition

result of HPP on glutamate level was similar to several AEDs that have been reported to reduce glutamate release. Drugs, such as lamotrigine or riluzole, reduced glutamate release by blockade of Na^+ channels. *In vitro* experiments with Na^+ channel blockers, carbamazepine and oxcarbazepine, have shown that these drugs inhibit veratrine-induced glutamate release from rat brain slices at therapeutically relevant concentrations (Waldmeier et al., 1996). Also in recently reports, as a voltage-gated sodium channel blocker, VPA could modulated sodium channels in the CNS to influence glutamate release (Rowley et al., 1995). Thus, inhibitory mechanism of VPA on the level of excitatory neurotransmitters is worth being further investigated.

Based on the study using patch-clamp technique, it was demonstrated that the anticonvulsant mechanisms of HPP is unlikely to involve potentiation of $GABA_A$, glycine or inhibition of NMDA receptors. Concentration of 0.1-300 μM HPP could not potentiate the $GABA_A$ currents induced by 3 μM GABA. HPP at a concentration up to 300 μM did not affect the glycine-induced inward current. These results were in line with preliminary study that HPP was ineffective in bicuculline (a competitive $GABA_A$ antagonist) and strychnine (a competitive glycine antagonist) convulsion test (Supatchaipisit, 1995). Thus, it is apparent that HPP could not modulate post-synaptic inhibitory neurotransmission in *in vitro* rat hippocampal neurons. Though, we found an inhibition of the pre-synaptic excitatory glutamate neurotransmission resulting from the acute administration of HPP in freely moving rats, HPP could not alter excitatory NMDA-induced inward current in this study and indicating that, HPP could not inhibit function of NMDA receptor.

Based on the result that HPP, in the dose dependent manner, significantly decreased the level of glutamate, it was likely that the decrement of glutamate could be the primary mechanism underlying anticonvulsant effect observed in rats. However, mechanisms of glutamate inhibition remain to be further investigated. Several mechanisms may account for inhibition of pre-synaptic glutamate neurotransmission such as those involved in the cycle of synthesis and uptake of glutamate. In addition, several AEDs have been reported to reduce glutamate release,

and it was concluded that this effect might be more indicative of their actions on neuronal Ca^{2+} and Na^{+} channels than a direct effect on the glutamate system (Stefani et al., 1997). Whether this explanation is applicable for HPP remains to be further investigated.