Chapter IV

Discussion and conclusion

The maximal electroshock (MES) and the pentylenetetrazol (PTZ) seizure tests are the most commonly used models for the evaluation of anticonvulsant activity, because they are simple and highly reproducible. The efficacy in MES test has been shown to correlate with an ability to prevent partial and generalized tonic – clonic seizures while PTZ test evaluates the ability of compounds to prevent absence and myoclonic seizures. Furthermore, in addition to clinical efficacy of drugs results from these models provide preliminary clues to mechanism of action as well (Rogawski and Porter, 1990, Loscher et al., 1991).

In the present studies, VHA and VPA demonstrated anticonvulsant activity in both MES and PTZ seizure tests. Similarity in anticonvulsant profile exhibited by VHA and VPA suggests that VHA 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. Furthermore, VHA was more effective than VPA in MES test and was as effective as VPA in PTZ test. The median effective dose (ED_{50}) of VHA were 114 and 97 mg / kg B.W. in MES and PTZ tests respectively while corresponding values for VPA were 211 and 99 mg / kg B.W. (Table 4). Based on the observation that the optimal pretreated time of VHA and VPA (i.p.) were 15 min and 30 min respectively (Figure 7), it is apparent that VHA acts more rapidly than VPA. Amide existing in the structure of VHA was believed to be minimally bound to plasma protein resulting in a better penetration into the brain than their corresponding acids (Bialer et al., 1994). Therefore, a shorter onset of action was demonstrated by VHA. However, further detail on the pharmacokinetic profile of VHA remains to be resolved.

Anticonvulsant activity of VPA in MES and PTZ tests previously described is in agreement with those having been reported from this laboratory (Pornchuree Supatchaipisit, 1995; Wandee Yeamvanichanun, 1997) and by other investigator (Pinder et al., 1977; Ferrendelli et al., 1989).

Peak effect of VHA was observed at 15 min (ED_{50} = 114 mg / kg B.W.) while peak effect of VPA was observed at 30 min (ED_{50} = 211 mg / kg B.W.) in MES test. Therefore, the maximal effect of VHA seemed to be more effective than VPA. However, its efficacy appeared to decline rapidly as a function of time. The ED_{50} of VHA at 30 min, 1, 3 and 6 hr after dosing were 148, 207, 466 and 832 mg / kg B.W., respectively whereas they were 218, 210 and 336 mg / kg B.W. at pretreated time of 1, 3 and 6 hr respectively for VPA. The short duration of action of VHA might be explained by pharmacokinetic properties of VHA from previous study which showed that the total clearance of VHA was 4 times larger than that of VPA, consequently, the half - life of VHA was shorter than that of VPA (Levi, Yagen and Bialer, 1997).

To prevent seizure attack, epileptic patients have to take anticonvulsant regularly. Therefore, an ideal antiepileptic should be orally active. In the present study, VHA was found to be orally active exhibiting the ED_{50} (242 mg / kg B.W. MES test) which was roughly two times higher than that of intraperitoneal administration (Table 4) indicating lower bioavailability of VHA when given orally. Like VPA (Table 4), poor aqueous solubility of VHA possibly accounts for higher oral ED_{50} observed. However, pretreated time of this experiment (VHA of 15 min, VPA of 30 min) was the optimal pretreated time of intraperitoneal administration. Thus, the absorbtion of oral administration might be uncomplete, consequently, the ED_{50} of oral administration was higher than that of intraperitoneal administration.

The fact that the enzyme systems responsible for the biotransformation of many drugs are located in the smooth endoplasmic reticulum and cytosol of the liver cells. These enzymes are also found in the other organs such as kidney, lung and gastrointestinal tract, but in small quantities (Gibson and Skett, 1994). Thus, the finding that VHA, was also active given 15 min before MES, when it was given by an intracerebroventricular route exhibiting the ED_{50} of 102 μM in comparison to the ED_{50} the of 132 μM of VPA. Furthermore, in degradation study, it is apparent that VHA was not degraded to VPA by either brain or liver homogenates (Figure 21 and 22). These results suggest that it was VHA per se that accounts for the anticonvulsant activity observed.

This findings are in line with the finding from previous study that VHA was not metabolized *in vivo* to VPA. Thus, the substitution of a hydrogen attached to the nitrogen in the VPA molecule by a hydroxyl moiety prevented the biotransformation of amide to VPA (Levi, Yagen and Bialer, 1997).

In combination with SKF-525A, one of the first alkylamines shown to elicit complexation with CYP, which has been widely accepted as a universal inhibitor of all CYPs except CYP_{2A1} in rat (Lin and Lu, 1998), It was found that the anticonvulsant potency of VHA and VPA were increased. The ED_{50} of VHA and VPA were 83 and 152mg / kg B.W. respectively. The finding of VPA is in line with the observation of Loscher (1981b). Generally, VPA is metabolized by several pathways. These include glucuronidation as a major pathway, β - oxidation and microsomal oxidation (ω -, ω_1-, ω_2- oxidation) (Zaccara et al., 1988). ω -, ω_1-, ω_2- oxidation, probably catalyzed by the microsomal CYP systems. Thus, inhibition of CYP by SKF-525A resulted in a decrease of ED_{50} of VPA which then suggested that VPA rather than its metabolite was the active agent (Granneman et al., 1981; Nau and Loscher, 1984). Like VPA, VHA may undergo several metabolic pathways and one of them is an inactivation catalyzed by the microsomal CYP systems as indicated by aforementioned results.

Effect of subchronic oral administration of VHA on its acute ability to reduce MES seizures (data shown in Figure 11) indicates that VHA retained its anticonvulsant potency in the MES seizure test following 5 days of daily oral dosing, suggesting minimal tolerance. A definitive conclusion would require longer periods of exposure. However, carbamazepine which induced its own metabolism could develop tolerance rapidly as its plasma clearance was more than doubles during the initial week of therapy (Anderson, 1998).

Bicuculline and strychnine seizure tests are the models used to probe the involvement of specific receptors. Bicuculline is a specific $GABA_A$ receptor antagonist and strychnine can block glycine receptor (Browning, 1991; Cooper et al., 1991). As shown in Table 4, the ED_{50} of VHA against bicuculline seizure test was 153 mg / kg B.W.,

thus VHA was more effective than VPA (ED_{50} = 382 mg / kg B.W.) in bicuculiine test. Comparatively VHA was less potent in strychnine test giving the ED_{50} of 441 mg / kg B.W. Therefore, an involvement of $GABA_A$ in anticonvulsant activity of VHA is very suggestive. Moreover, taking into consideration that the ED_{50} of VHA in strychnine test is much far apart from those in MES, PTZ and bicuculline tests (Table 4), an involvement of glycine receptors, if there is any, seems to be trivial. For VPA, the finding in bicuculline and strychnine tests is in line with the observation of Loscher (1985) and Ferrendeli et al. (1989), however disputed results on strychnine test have also been reported (Swinyard and Woodhead, 1982; Davis, Peter and McTavish, 1994).

For acute toxicity, the LD_{50} of VHA and VPA were 840 and 790 mg/kg B.W. respectively (Figure 15). Therefore, VHA appears to be slightly relevant than VPA with regards to LD_{50} values. Higher relative safety margin (LD_{50} / ED_{50}) than that of VPA was exhibited by VHA in both MES and PTZ tests, implying that VHA was able to offer greater safety than did VPA. However, in term of safety VHA seemed to be superior to VPA only within the first hour after dosing as the relative safety margin of VHA at 1, 3 and 6 hours were continuously decreased (Table 3).

Neurological signs, presumed to be CNS related such as ataxia, sedation, hypnosis, incoordination or tremors occurred after high doses of both VHA and VPA. These results are similar to the CNS related clinical signs of VPA in human (Walker et al., 1990).

Rotorod test of Dunham and Miya (1957) is the most commonly used screening test to estimate the minimal neurological deficit in experimental animal (Loscher, Noting and Fassbender, 1990). As shown in Figure 16, PEG 400 had no effect on rotorod test while VHA and VPA exhibited the median neurotoxic dose (TD_{50}) of 189 and 260 mg / kg B.W. respectively, resulting in protective indices (PI = TD_{50}/ED_{50}) of VHA in MES and PTZ tests of 1.66 and 1.95 respectively while the corresponding values of VPA were 1.23 and 2.63 respectively (Table 5). As it has been previously proposed that compound with an estimated PI at least 2 should be considered sufficient in MES or PTZ models

(Loscher and Nolthing, 1991). Thus, the present finding indicates that the PI of VHA is insufficient for antiepileptic drug evaluation and furthermore the PI of VHA at 1, 3 and 6 hours were decreased as a function of time (Table 6). For VPA, it was found that the PI is within the range of conventional antiepileptic drugs (Loscher and Nolting, 1991). Regarding to duration of neurotoxic effect, it is apparent that VHA demonstrated lower degree of neurotoxicity than VPA throughout the observation period of 6 hours (Figure 17),lower toxic of VHA may be due to rapid clearance of the test substance.

Determination of motor activity is considered to be the simplest method for detection of CNS sedative effect (Thompson, 1990). VHA and VPA in the median effective dose of the intraperitoneal route in MES and PTZ tests (Table 4) depressed locomotor activity to the same extent as did PEG 400 but not NSS which demonstrated a significant lower degree of depression (Figure 18). This indicates that the depression observed in VHA or VPA treated groups is substantially resulted from the vehicle used, PEG 400. Failure of VPA (100 and 200 mg / kg B.W. i.p.) to depress locomotor activity corresponds well with previous observation that VPA has no significant effect on locomotor activity at the dose levels which produce signs of neurotoxicity until dose of 500 – 600 mg / kg B.W. of VPA was reached (Pinder et al., 1977)

A tendency to depress CNS of PEG 400 was also demonstrated in barbiturate potentiation test. PEG 400 tended to prolong barbiturate sleeping time to an extent that a statistical significance from that of NSS was achieved (Figure 19). Like VPA, the low dose of VHA (90 mg / kg B.W.) did not prolong the barbiturate sleeping time whereas the high dose (120 mg / kg B.W.) did prolong it. This indicated that both VHA and VPA possess CNS depressant activity.

In conclusion, the present study demonstrated a potent anticonvulsant activity of VHA, by intraperitoneal route, in MES, PTZ as well as in bicuculline tests. However, VHA weakly blocked the effect of strychnine. A higher efficacy of VHA than VPA was demonstrated in all animal models tested except in PTZ test which VHA was as effective as VPA. In MES test, the duration of protection exhibited by VHA was shorter than VPA.

Like VPA, VHA was also orally active exhibiting the ED_{50} which was about 2 times higher than the ED_{50} by intraperitoneal route. Furthermore, VHA demonstrated higher protection against MES than did VPA when they were given by intracerebroventricular route. In combination with SKF-525A (a P450 enzyme inhibitor) the anticonvulsant potency of VHA and VPA were increased. In addition, VHA did not display any signs of tolerance following 5 days of daily oral dosing.

VHA possessed a higher relative safety margin (LD_{50} / ED_{50}) than did VPA in the first hour after dosing. In parallel with its efficacy, the median effective dose of VHA seemed to elicit the same degree of neurological deficit as did VPA in rotorod test giving the protective index ($PI = TD_{50} / ED_{50}$) of 1 – 2. Moreover, no depressant effect of VHA and VPA was observed in locomotor activity test, while it was apparent in barbiturate sleeping time test. Degradation studies demonstrated that VHA was not degraded to VPA either in brain or liver homogenates.

Taken together, a novel derivative of valpromide, VHA, demonstrated better anticonvulsant activity with approximately the same profile of toxicity as VPA. VHA was orally active and did not display tolerance potential. However VHA exhibited a short-lived anticonvulsant activity which should be further improved by structural modification.