Chapter v

Discussion and Conclusion

One electrical and three chemical tests were used in an initial screening for anticonvulsant activity of a new valproic acid analogue, HMV, in comparison with its parent compound, VPA. The MES and PTZ tests were selected on the ground that they are the most commonly used models and highly reproducible. Furthermore, results in these two models contribute clues to clinical efficacy as well as possible mechanisms of the test compounds (Rogawski and Porter, 1990). The MES is used as a model for generalized tonic-clonic seizures whereas seizure induced by a subcutaneous administration of PTZ is used as a model for generalized seizure of the petit mal type (Loscher et al., 1991). Another two chemical tests, bicuculline and strychnine, were added in order to probe the involvement of $GABA_A$ and Glycine receptors respectively (Browning, 1991; Cooper et al., 1991).

The results of the present studies demonstrate that HMV is a relatively potent, rapidly acting and orally effective anticonvulsant in MES and PTZ tests (Figure 5 ; Table 1). In both models, the ED_{50} of oral administration of HMV are roughly two times higher than those of intraperitoneal administration (Table 1) indicating lower bioavailability of HMV when given orally. Like VPA (Table 1), poor aqueous solubility of HMV may partly account for the result obtained. On the other hand, lipophilic properties of HMV may facilitate the penetration through the blood-brain barrier. Amides existing in the structure of HMV 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 HMV (Figure 5). However further detail on the pharmacokinetic profile of HMV remains to be resolved.

As described in result (1.3), HMV is rather ineffective in convulsive models induced by an intraperitoneal injection of glycine receptor antagonist (strychnine) or $GABA_A$ receptor antagonist (bicuculline) (Browning, 1991; Cooper et al., 1991). Therefore it is very suggestive that the anticonvulsant activity of HMV observed is not directly related to glycine or $GABA_A$ receptors. However, based on the result that HMV was highly active (ED_{50} = 35 mg/kg B.W. i.p.) against PTZ which is generally known to diminish GABA's inhibition indirectly, any GABA-related effects other than direct effect at $GABA_A$ receptor site could be involved.

In accordance with previous work from this laboratory (Chatchai Powthongchin, 1994; Thongchai Sooksawate, 1995) and other investigators (Pinder et al., 1977; Ferrendelli et al., 1989), VPA exerted a similar anticonvulsant profile but in higher dose than those elicited by HMV. Approximately, the ED_{50} of VPA was 3 times higher than its counterpart in the same experimental condition (Table 1). Therefore, it can be stated that in addition to a shorter onset, HMV is at least 3 times more potent than VPA in all models tested.

As shown in Figure 6, 7 despites almost equal LD_{50} (722 and 717 mg/kg B.W. i.p. for HMV and VPA, respectively), HMV appears to be about 3 time safer than VPA if the relative safety margin (LD_{50}/ED_{50}) was taken into account (Table 2). With reference to the results that the ED_{50} of HMV (i.p.) was generally only one third of

the one elicited by VPA i.p., this finding is not surprising. Hence, HMV is predicted to have a greater margin of safety than VPA in clinical use.

At therapeutic doses, an ideal anticonvulsant should have no unwanted effects such as sedation, impairment of motor function and other adverse effects (Rall and Schleifer, 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). On the contrary to NSS and PEG 400 which had no effect on test behaviour, HMV and VPA (i.p.) exhibited the median neurotoxic dose (TD_{50}) of 89 and 274 mg/kg B.W. respectively (Figure 7; Table 2). However, both of them demonstrated a similar range of the protective index (PI = TD_{50}/ED_{50}) of about 1-2 (Table 2). Resemble results of VPA was formerly reported by Loscher and Nolting (1991) who have calculated the PI of various standard antiepileptics currently used and concluded that a PI of 2 should be considered sufficient in MES or PTZ models.

Determination of motor activity is considered to be the simplest method for detecting CNS sedation effects (Thompson, 1990). HMV and VPA in the median effective dose of the intraperitoneal route in MES and PTZ tests (Table 2) depressed locomotor activity to the same extent as did PEG400 but not NSS which demonstrated a significant lower degree of depression (Figure 9, 10). This indicates that the depression observed in HMV or VPA treated groups is substantially resulted from the vehicle used, PEG400. Failure of VPA (100 and 200 mg/kg B.W. i.p.) to depress locomotor activity corresponds well with previous works reporting that VPA in the dose range of 10-400 mg/kg B.W. i.p. had no significant effect on locomotor activity (File and Aranko, 1988) and no evidence of behavioural effect was noted until the doses of 500-600 mg/kg B.W. of VPA was reached (Anlezark et al., 1976).

A tendency to depress CNS of PEG400 was also demonstrated in barbiturate potentiation test. PEG400 tended to prolong barbiturate sleeping but not to an extent that a statistical significance from that of NSS was achieved (Figure 11). However, the difference between PEG400 and NSS was revealed when VPA (100 mg/kg B.W.) was shown to prolong barbiturate sleeping time significantly from those elicited by NSS but not PEG 400. As illustrated clearly in Figure 11 that low dose of HMV (35 mg/kg B.W.) did not prolong the barbiturate sleeping time whereas the higher one (75 mg/kg B.W.) did prolong it to the same extent as did 200 mg/kg B.W. of VPA. Thus, a small degree of sedation or, at the most, being as sedate as VPA is expected to accompany the therapeutic dose of HMV.

In an attempt to investigate the effect of the test substance on brain GABA, microdialysis technique which allows the continuous observation was used to detect the effect of HMV and VPA on cortical GABA of the rats.

The spontaneous release of GABA gradually decreased with time. After the intraperitoneal administration of NSS the amounts of GABA collected in the third hour was only 56% of its first hour value (Figure 14). Depletion due to long time perfusion may account for the decrement observed. In order not to overlook the alteration of spontaneous release of GABA which might be minimal at certain points as well as the fluctuation of the detection value (Figure 15), changes of the total amount of GABA over a period of 180 min were used to evaluate the effects of test substances. As shown in Figure 14 and 15, no statistical significance was observed between NSS and PEG400 treated groups. However, based on the result that the amount of GABA in PEG400 treated group collected at the third hour was about the same as its first hour value in relation to NSS, PEG400 seems to increase cortical GABA to a minor degree. This result is in line with and may explain the depression

effect of PEG400 observed in locomotor activity test (Figure 9 and 10) as well as the potentiation of barbiturate sleeping time (Figure 11), however, this has to be proved by further investigation.

Higher doses of HMV and VPA were used in this experiment so that the effects on GABA level, if there is any, was magnified. VPA 200 as well 400 mg/kg B.W. significantly increased the amount of GABA. Conflicting results that VPA 200 and 400 mg/kg B.W. had no effect on cortical GABA were reported from this laboratory (Chatchai Powthongchin, 1994; Thoungchai Sooksawate, 1995), however it should be noted that different method of evaluation was used to assess the effect of VPA. Point to point comparison was made in the previous reports whereas the present studies compared total change of GABA. This may account for the difference observed. Accordingly, inconsistent results of VPA also exist between various laboratories. VPA was reported to increase brain and synaptosomal GABA concentration by a number of investigators (Godin, et al, 1969; Simler et al., 1973; Iadarola and Gale, 1979; Loscher and Vetter, 1985; Biggs et al., 1992) whereas some investigators fail to demonstrate such effect (Anlezark et al.,1976). However, it is generally accepted that more than one mechanism is responsible for antiepileptic activity of VPA (Zona and Avoli, 1990).

Apparently, HMV 75 mg/kg B.W. did not have significant effect on cortical GABA whereas HMV 150 mg/kg B.W. did increase it. In comparison to VPA which significantly increased brain GABA when given in the doses of 200 and 400 mg/kg B.W., it is unlikely that an increment of brain GABA is the principal mechanism responsible for the anticonvulsant activity of HMV. However, like VPA, HMV may possess a wide spectrum of mechanism of action in which an augmentation of

GABA, seen in the dose of about 2 times higher than its ED_{50}, is one among them. Other studies on its possible mechanisms remain to be explored.

In conclusion, the present studies have demonstrated a potent anticonvulsant activity with rapid onset of HMV. Like VPA, HMV is orally effective and its potency is at least about 3 times higher than that of VPA. Similar results obtained in safety evaluation in which HMV exhibited a relative safety margin (LD_{50}/ED_{50}) that is also 3 times higher than that of VPA. In parallel with its efficacy, the median effective dose of HMV 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. No depressant effect of the ED_{50} of HMV and VPA was observed in locomotor activity test, however this effect of VPA was noted in the potentiation of barbiturate sleeping time. With regards to possible mechanisms of HMV, results from microdialysis technique suggest that an augmentation of brain GABA is unlikely to be a principal mechanism but only one among many other mechanisms underlying anticonvulsant activity observed and they remain to be investigated.