Chapter IV

Discussion and conclusion

MES was the model widely used to evaluate anticonvulsant activity. Efficacy in this model has been shown to correlate with ability to prevent partial and generalized tonic clonic seizure in man, and it is often stated that this model evaluated the capacity of drug to prevent the spread of seizure discharge. Furthermore, result in this model provide preliminary clues to the mechanism of action as well as clinical efficacy of drugs (Rogawski and Porter, 1990).

In accordance with previous work from this laboratory (Thongchai Sooksawate, 1995), the present studies demonstrated a greater potency of an intraperitoneally given VPU (ED_{50}= 71 mg/kg B.W.) than those of VPA (ED_{50}= 199 mg/kg B.W.) in protection against MES in mice. The preferable efficacy of VPU was also noted when it was given orally. As shown in Figure 13, the ED_{50} of VPU were 413, 293 and 215 mg/kg B.W. at pretreated time of 30, 60 and 120 min respectively while the corresponding values of VPA were 1341, 512 and 522 mg/kg B.W. This finding is in favor of VPU as it is generally accepted that an ideal antiepileptic drug must be orally effective (Swinyard and Woodhead, 1982). Like VPA, poor aqueous solubility of VPU possibly accounts for higher oral ED_{50} observed. However, many more experiments must be accomplished in order to achieve a well defined pharmacokinetic characteristics of this compound.

Regarding to duration of anticonvulsant activity, it is apparent that intraperitoneal administration of either VPU or VPA exhibited protection against

MES at least until 12 hours after dosing, however, with an increment of the ED_{50} values (Table 2). As illustrated in Figure 10, the ED_{50} of VPU were always lower than those of VPA at any given time. This finding confirms the higher potency of VPU than VPA throughout the observation period. The sharp rise of ED_{50} of VPU as well as that of VPA were noted at time between 3-6 hours after dosing and then the ED_{50} gradually rised until the last point of observation at 12 hours. Thus, it is likely that, resemble to VPA which demonstrated the time to peak plasma level within 1-4 hours and being metabolized extensively before its elimination (Adkison et al., 1995), VPU may undergo through similar fate. However, as the ED_{50} of VPU and VPA at 12 hours were approximately 6 and 3 times higher than their corresponding ED_{50} at first hour, it is suggestive that VPU might be metabolized faster than VPA. This assumption is highly speculative since nothing is known about the metabolism of VPU except the finding from the present studies that VPU were not degreaded to VPA by either brain or liver homogenates (Figure 17 and 18).However, the results from degradation studies did suggest that it was VPU *per se* and/or any other of its metabolizes but not VPA accounts for the anticonvulsant activity observed.

The fact that the ED_{50} of VPU and VPA in the present studies were almost identical with those previously reported by Thongchai Sooksawate (1995), renders the determination of the relative safety margin of the test substances in the present studies in relation to their previously reported LD_{50}. As shown in Table 2, the relative safety margin (LD_{50}/ED_{50}) of VPU and VPA at 1, 3, 6, 9, and 12 hours were decreased as a function of time. However, in comparison to VPA, a greater relative safety margin was always exhibited by VPU. Therefore, in terms of safety VPU seems to be superior to VPA.

Most antiepileptic drugs suffer from a broad range of undesirable side effects such as sedation, cognitive disorder, 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). As shown in figure 15, VPU in the dose approximately 6 times higher than those needed to protect against MES, inhibited rotorod performance. At pretreated time of 30 min, the median toxic dose (TD_{50}) of intraperitoneally given VPU and VPA were 625 and 265 mg/kg B.W. respectively resulting in neuroprotective indices ($PI=TD_{50}/ED_{50}$) of 8.8 for VPU and 1.3 for VPA (Table 3). As it has been previously proposed that compound with an estimated PI of at least 2 in MES model should proceed to further evaluation (Loscher and Nolting, 1991), the present finding substantiates such opportunity for VPU. For VPA, it was found that the PI is not only well within the range of conventional antiepileptic drugs (Stagnitto et al., 1990; Loshcher and Nolting, 1991) but also in good agreement with those previously reported from this labolatory (Ponchulee Supatchipisit, 1995) and other investigators (Loscher and Nolting, 1991). A substantially higher value of PI demonstrated by VPU implys that VPU may have a reduced potential for neurological side effects in human. This view was further elaborated by the finding that VPU (300 and 400 mg/kg B.W.) demonstrated a minor degree of neurotoxicity throughout the observation period of 12 hours (Figure15). Therefore it can be anticipated that high doses of VPU could be given clinically to achieve a complete control of epilepsy without a serious side effect on motor coordination.

Imbalance between excitatory and inhibitory amino acid neurotransmitter has long been believed to be major factor in genesis of convulsion disorder (Davidoff, 1983; Rogawski and Porter, 1990). Inhibition of excitatory transmission

and/or potentiation of inhibitory transmission have been demonstrated to be the possible mechanism of a number of currently used antiepileptic drugs (Graves and Leppik, 1991) including VPA which has been shown to increase GABA concentrations in whole brain (Godin et al., 1969) and synaptosomes (Loscher et al., 1981; Loscher and Vetter, 1985). Significantly , VPA appears to preferentially enchance GABA turnover in neuronal compartment (Ladarola and Gale, 1981) and thus might be expected to increase GABA ergic transmission. In vitro evidence suggesting that VPA enhanced of cerebral GABA synthesis was recently provided by Silverman et al., (1991). Although Farrent and Webstar (1989) failed to observe any change in sapontaneous nigral GABA release by VPA using push-pull cannulae. In line with previous studies on ventral hippocampus of conscious freely moving rats where sodium valproate had no effect on aspartate whereas an increase in GABA level was noted after high dose of valproate (400 mg/kg B.W.) was given, similar results were observed in the present studies (Biggs et al., 1992). A dose-dependent increase in GABA level seems to account for anticonvulsant activity of VPA(Figure 31). However, this dose not exclude other mechanisms as it was evident in the present studies that VPA (400 mg/kg B.W.) significantly increased the level of cortical glycine (Figure 30). As perplexing results of VPA was seen on glutamate level which being decreased only by low but not high dose of VPA. (Figure 29). All these finding are in accordance with the proposal that VPA exerts its anticonvulsant effect through a combination of several mechanisms including those related to amino acid neurotransmitters (Davis, 1994).

Surprisingly, VPU was reported to decrease both the excitatory and inhibitory cortical amino acid neurotransmitter in anesthetized rats (Thongchai Sooksawate, 1995). Since the reduction was most prominent on glutamate and being least on glycine, it was suggested that this finding might, in part, contributed

to the anticonvulsant activity. Additionally synergism between VPU and the anesthetic used, pentobarbital was proposed to be responsible for the non selective depression observed.(Thongchai Sooksawate, 1995).

Different profile of responses were noted in conscious freely moving rats in the present studies. However, it should be pointed out that current comparison was made on the total change of amino acid over a period of 180 min whereas point to point comparison was made at every 20 min time interval in the previous studies.

As shown in Figure 20, 21, 22 and 23, no statistically difference on the level of aspartate, glutamate, glycine and GABA was 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 VPU and VPA treated groups.

In contrast to the observation in anesthetized rats, VPU did not exert any depressant effect on the level of cortical aspartate, glycine and GABA in awake rats (Figure 28, 30 and 31). A reduction of glutamate was noted in VPU treated group (Figure 29), however, only in the low dose (200 mg/kg B.W.) while high dose (400 mg/kg B.W.) had no effect. Therefore, a prominent dose dependent reduction in cortical glutamate level in anesthetized rats is most likely to be resulted from synergistic effect between VPU and pentobarbital rather than the effect of VPU alone. Furthermore this finding also rules out an inhibition of glutamate as a major mechanism underlying anticonvulsant activity of VPU as its activity apparently was non dose dependent. However, contribution of this mechanism as a subordinate mechanism especially for VPU low dose is conceivable. Unfortunately, we cannot

offer any explanation for deprivation of glutamate depressant effect of high dose VPU in the present studies.

In addition, the disparity between the effects of VPU on the level of cortical aspartate, glutamate and glycine in anesthesized and awake rats did support previous hint that the non-selective depressant effect of VPU on both the excitatory and inhibitory amino acid neurotransmitters in anesthesized rat was due to synergism of VPU and pentobarbital (Thongchai Sooksawate, 1995). The results of VPU reported herein suggests that, despite being an ureide analog of VPA, VPU appeares to differ from VPA with regards to mechanism of action. VPU seems to exert its anticonvulsant activity by any machinery other than GABA ergic mechanism which is generally accepted as a principal mechanism of anticonvulsant activity exhibited by VPA.

In conclusion the present studies confirm the greater anticonvulsant activity with lower toxicity of an intraperitoneally given VPU in comparison to VPA as previously reported by Thongchai Sooksawate (1995) and this observation holds true for at least 12 hours after dosing. VPU is also orally active giving the ED_{50} which was about 6 times higher than its corresponding value by intraperitoneal route. Degradation studies demonstrates that VPU was not degreaded to VPA either by brain or liver homogenates. Therefore it is VPU *per se* and/or any of its metabolite other than VPA that accounts for the anticonvulsant activity observed. Further studies by microdialysis technique in awake rats revealed different profiles of responses on cortical amino acid neurotransmitters exhibited by VPU and VPA. GABA ergic mechanism as well as glycine appear to be responsible for the anticonvulsant activity of VPA but not VPU which had no effect on GABA, glycine and aspartate level. The finding that glutamate level was inconsistently

reduced by different dose of VPU excludes the possibility of being major mechanism underlying anticonvulsant activity of VPU which remains to be further elucidated.

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