Chapter IV

Discussion

MES and PTZ seizure models are the most widely used screening test for antiepileptic drugs because of their high reproducibility. Furthermore, results in these two models provide preliminary clues to the mechanism of action as well as clinical efficacy of the drugs (Rogawski and Porter, 1990). It is often stated that drugs that are active in MES test have a phenytoin-like effect on voltage-dependent Na^+ channels and most likely to be effective in generalized tonic-clonic (grand mal) seizure (Rogawski and Porter, 1990; Loscher et al., 1991). On the other hand, anticonvulsant activity in PTZ test suggests the action on GABAergic neuronal system and may correlate well with activity against absence seizure (Rogawski and Porter, 1990; Loscher et al., 1991). In addition, the drug interfering with specific receptors can also be identified by using receptor antagonists such as bicuculline, a specific $GABA_A$ receptor antagonist, or strychnine which block glycine receptor (Ticku and Rastogi, 1986; Ferrendelli et al.,1989).

In the present studies, like VPA, VPU demonstrated a broad spectrum anticonvulsant activity in MES, PTZ and bicuculline tests (Table 3) but they were ineffective in strychnine test (Table 3). Quatitatively, VPU appears to be equally effective in MES and PTZ tests whereas VPA tended to be more effective in PTZ test. Contribution of species difference between mice and rats in responses observed is mostly minor, none in MES test and to a small extent in PTZ test (Table 3). Results of VPA in the present studies are in agreement with those previously reported from this laboratory (Chatchai Powthongchin, 1994) and by other investigators (Swinyard and Woodhead, 1982; Clark, 1988). Despite of the same spectrum of activity, the anticonvulsant activity of VPU was generally displayed with doses which are lower than those of VPA (Table 3). In this respect, VPU seems to be approximately 4 times and 2 times more effective than VPA in MES and PTZ tests respectively.

In seizure models using inhibitory neurotransmitter receptor antagonists bicuculline and strychnine, VPA and VPU were relatively less potent in bicuculline test and were ineffective in strychnine test (Table 3). Thus, it is tempting that intervention of glycine receptor does not account for anticonvulsant activity of VPU. Moreover, taking into consideration that the ED_{50} of VPU in bicuculline test is much far apart from those in MES and PTZ tests (Table 3), an involvement of $GABA_A$ 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 Ferrendelli et al. (1989), however, disputed results on strychnine test has also been reported (Swinyard and Woodhead, 1982; Clark, 1988).

Toxicity testing in term of lethality and neurotoxicity indicate a promissing prospect for VPU. As illustrated in Table 4, the LD_{50} of VPU is somehow about 20-30 times higher than its ED_{50} in MES and PTZ tests whereas VPA respectively demonstrated a difference of only 4-10 times. The observed neurotoxic signs such as ataxia, sedation and hypnosis presumably related to CNS occured only after high dose of VPA and VPU were given and they are similar to the CNS-related clinical signs of VPA in human (Walker et al., 1990).

Regarding to the effect on barbiturate sleeping time, at the dose of 100 mg/kg B.W., VPU significantly prolonged pentobarbital sleeping time to the same extent as did VPA. However, a higher degree of potentiation than that of VPA was elicited by VPU at the dose of 200 mg/kg B.W. (Figure 15).

It is noteworthly to point out that regardless of results of bicuculline test, the ED_{50} of VPA in mice was 242 and 95 mg/kg B.W. in MES and PTZ tests

respectively whereas they are 66 and 57 mg/kg B.W. for VPU in the same seizure models (Table 3). Therefore it is not surprising to note a higher degree of depression of VPU than VPA when the dose of 200 mg/kg B.W., roughly equal to the ED_{50} of VPA but 3 times higher than the ED_{50} of VPU, was applied. Taking into the consideration that the same degree of barbiturate potentiation was exhibited by VPA and VPU at the dose of 100 mg/kg B.W. (Figure 15), a lower degree of CNS depression is expected for VPU from the therapeutic point of view. Together with its comparatively high LD_{50} (Table 4) VPU appears to offer a higher margin of safety than VPA.

A disturbed balance between excitation and inhibition input is believed to be a major factor in the genesis of convulsion disorders (Davidoff, 1983; Rogawski and Porter, 1990). Therefore, a microdialysis technique which allows a continuous monitoring of the extracellular levels of various compounds in the brain was used to investigate the effects of VPA and VPU on the levels of cortical excitatory (glutamate and aspartate) as well as inhibitory (glycine and GABA) amino acid neurotransmitters in anesthetized rats.

Among the four amino acid neurotransmitters under investigation, only aspartate was found to be significantly decreased in a dose dependent manner after the administration of VPA (Figure 17-20). Similar results of VPA on aspartate have been previously reported by many investigators (Chapman, Croucher, and Meldrum, 1984; Thurston and Hauhart, 1989) but a body of controversial results did exist for both glutamate and GABA (Simler et al., 1973; Anlezark et al., 1976; Loscher and Vetter, 1985;Davis, Peters, and McTavish, 1994). For example, VPA has been reported to increase concentration of brain GABA by Simler et al. (1973) and Loscher and Vetter (1985) while other studies failed to demonstrate the elevation of GABA

(Anlezark et al., 1976). As previously reported, synaptically released GABA is inactivated by reuptake into glia and neurones especially inhibitory interneurones (Iversen and Kelly, 1975), if reuptake mechanism of GABA is greater than diffussion into microdialysis probe, the results from microdialysis studies should fail to demonstrate the elevation of extracellular GABA. Furthermore the possibility that the anesthetic used, pentobarbital, can reduce the Ca^{2+}-dependent release of neurotransmitter (Rall, 1990; Ticku and Kulkarni, 1992). These may result in a failure to demonstrate the elevation of extracellular GABA level by VPA in the present studies. However, whether changes in one or more of these brain neurotransmitters account for anticonvulsant activity of VPA remain to be elucidated. At present, it is generally accept that VPA is an antiepiletic drug with a wide spectrum of mechanism of action (Rogawski and Porter, 1990; Tunnicliff, 1991).

In contrast, VPU significantly decreased cortical levels of aspartate, glutamate, glycine and GABA in a dose dependent manner (Figure 17-20). Glutamate was the most whereas glycine was the least sensitive to the effect of VPU (Table 5 and Figure 18 and 19). Normally, a reduction of inhibitory neurotransmitter, usually leads to a convulsive response (Horton, 1991). Therefore, it is very unlikely that the reduction of glycine and GABA is responsible for the anticonvulsant activity of VPU. However if this effect was encountered by a stronger reduction of excitatory neurotransmitter, an anticonvulsant activity would then be accomplished. This may be the case for VPU which decreased levels of both excitatory and inhibitory neurotransmitters but the reduction was most prominent on glutamate and being least on glycine (Table 5 and Figure 18 and 19). A stronger correlation between the anticonvulsant activity exhibited by phenobarbital (Gage, McKinnon, and Robertson, 1986) as well as a series of valproate analogues (Chapman, Meldrum, and Mendes, 1983; Chapman, Croucher, and Meldrum, 1984) and

their ability to block or reduce cerebral excitatory transmission has been reported. The facts that the structure of VPU was modelled to contain acyclic major part of barbiturate and barbiturate can depress Ca^{2+}-dependent action potentials as well as reduce the Ca^{2+}-dependent release of neurotransmitter (Rall, 1990; Ticku and Kulkarni, 1992), may contribute to a non selective depression of all neurotransmitters observed in the present studies. Thus, a stronger synergistic effect of VPU with pentobarbital, the anesthetic used, is questionable and essential to be further clarified in concious rats. However, due to the finding that VPU was active in various models of seizure, it is very suggestive that, like VPA, VPU possesses a variety of pharmacological actions that may account for its anticonvulsant activity and they remain to be identified.