Chapter IV

Discussion

Because of their high reproducibility,two standard anticonvulsant screening tests, the maximal electroshock (MES) and the pentylenetetrazol (PTZ) seizure tests are mostly used to evaluate anticonvulsant activity. Furthermore, the MES and PTZ seizure tests exhibit markedly different pharmacologic profiles. The efficacy in MES test has been shown to correlate with the ability to prevent partial and generalized tonic clonic seizures while PTZ test evaluates the ability of compounds to prevent absence and myoclonic seizures. Moreover, results in these models provide preliminary clues to the mechanism of action as well as clinical efficacy of drugs (Miller et al, 1986; Rogawski and Porter, 1990; White, 1997).

The present study reported herein anticonvulsant activity of the newly synthesized valproic acid analog, Amide 1C, in both MES and PTZ seizure tests. Similarity in anticonvulsant profile exhibited by Amide 1C and VPA suggests that Amide 1C 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, it is conceivable that Amide 1C is more potent than VPA in both MES and PTZ seizure tests. Results of VPA in the present study are in agreement with those previously reported from this laboratory (Thongchai Sooksawate, 1995; Pornchulee Supatchaipisit, 1995) and by other investigators (Ferrendelli, 1989; Shuto and Nishigake, 1979 and Pinder et al, 1977). Based on the observation that the optimal pretreated times of Amide 1C and VPA were 15 and 30 min respectively (Table 3.), it is apparent that Amide 1C acts more rapidly than VPA. This might also indicate the highly lipophilic nature of Amide 1C which could facilitate the penetration through blood brain barrier better than did the VPA. Amide 1C was also orally active. Though, the ED_{50} of orally given Amide 1C cannot be determined due to shortage of supply of the test substance, Amide 1C in the dose of 400 mg/kg BW., given orally, was able to give a complete protection against MES at pretreated time of 15 min in all mice (n = 4) tested (result

1.1.2). therefore, it can be concluded that Amide 1C is an orally active substance which is rapidly absorbed from the GI tract.

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 (Ticku and Rastogi, 1986; Ferrendelli et al, 1989 and Cooper et al, 1991).

The data obtained from models using respective antagonists demonstrated that Amide 1C is more effective than VPA in bicuculline seizure test but is ineffective against strychnine test as VPA (Table 3.). Antagonism of Amide 1C at glycine receptor can be ruled out by the negative effect in strychnine test. However an involvement of $GABA_A$ is very suggestive as illustrated by the ED_{50} of 214 mg/kg BW. of Amide 1 C in the protection against bicuculline-induced convulsion (Table 3.). Taken together with the finding that the ED_{50} of Amide 1C in MES model is only 81 mg/kg BW., it can be anticipated that antagonism at $GABA_A$ receptor could be, at the most, partially attributable to the anticonvulsant activity exerted by Amide 1C. Any other mechanisms besides antagonism at $GABA_A$ receptor should take part as well and they remain to be elucidated. 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; Davis, Peters and McTavid, 1994).

Toxicity in terms of lethality and neurotoxicity of Amide 1C in comparison to VPA was summarized in Table 4. The LD_{50} of Amide 1 C and VPA when given intraperitoneally were 602 and 832 mg/kg BW. respectively. Though Amide 1C appears to be less relevant than VPA with regards to LD_{50} values, a higher relative safety margin (LD_{50}/ED_{50}) than that of VPA was exhibited by Amide 1C in both the MES and PTZ tests. Therefore, at the median effective dose Amide 1C was able to offer greater safety than did VPA.

Neurological signs, presumed to be CNS related, such as ataxia propagation, sedation, hypnosis, incoordination or tremors are occurred after high doses of both Amide 1C 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) was widely used to assess the minimal neurotoxicity of anticonvulsant. As illustrated in Figure 13., PEG400 had no effect on rotorod test while Amide 1C and VPA exhibited a dose dependent inhibition of rotorod performance (Figure 14.). Results were expressed as the median neurotoxic dose (TD_{50}) in Table 4. Though the TD_{50} of Amide 1C and VPA were comparable (313 and 386 mg/kg BW. respectively) the protective indices ($PI = TD_{50}/ED_{50}$) of Amide 1C in both MES and PTZ tests were about 2 times higher than those of VPA (Table 4.).

The PI value is widely used in primary and secondary screening for drugs with selective anticonvulsant activity. The Antiepileptic Drug Development Program of Epilepsy Branch of NIH suggested that the PI of at least 5 has been proposed to be a critirion for drug that should proceed to further evaluation. Besides, it should be considered that in human the margin between anticonvulsant dose and dose exerting sedative, ataxia or other neurotoxic adverse effects is less than 5 for all the common drugs including VPA, so that a criterion of 2 is probably sufficient for anticonvulsant drug evaluation (Loscher et al, 1991). Thus, Amide 1C should be favorably selected for further evaluation.

Determination of motor activity is considered to be the simplest method for detecting CNS sedative effects (Thompson, 1990). Amide 1 C and VPA in the median effective dose of the intraperitoneal route in MES and PTZ tests (Table 3.) depressed locomotor activity to the same extent as did PEG 400 but not NSS which demonstrated a significant lower degree of depression (Figure 15.). This indicated that the depression observed in Amide 1C or VPA treated groups is substantially resulted from the vehicle used, PEG 400. Failure of VPA 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 the dose of 500-600 mg/kg BW. of VPA was reached (Pinder et al, 1977).

Potentiation of barbiturate sleeping time was used to evaluate the CNS depression of test substances. As illustrated in Figure 16., like VPA, the low dose of Amide 1C (50 mg/kg BW.) did not prolong the barbiturate sleeping time whereas the high dose (100 mg/kg BW.) did prolong it. This indicated that both Amide 1C and VPA possess CNS depressant activity.

Imbalance between excitatory and inhibitory amino acid neurotransmitters has been believed to be a major factor in genesis of convulsion disorders (Davidoff, 1983; Rogawski and Porter, 1990). To evaluate the effect of Amide 1C and VPA on brain amino acid neurotransmitters, microdialysis technique was used to measure the extracellular amino acid neurotransmitters, namely, aspartate, glutamate, glycine and GABA, in the cerebral cortex of anesthesized rats.

As shown in Figure 19 and 20, no statistically significant difference in the level of aspartate, glutamate, glycine and GABA was observed between NSS and PEG 400 treated groups. The low dose of Amide 1C (100 mg/kg BW.) tended to reduce brain aspartate but not to the extent that a statistical significance from that of PEG 400 was achieved (Figure 21.) Amide 1C produced no significant changes in basal levels of cerebral cortex aspartate, glutamate, glycine and GABA (Figure 21, 22, 23 and 24). At doses of 200 and 400 mg/kg BW., VPA did not significantly alter basal GABA levels . The same results were reported from this laboratory, (Chatchai Powthongchin, 1994; Thongchai Sooksawate, 1995), and some investigators (Anlezark et al, 1976). While other investigators had demonstrated that VPA could elevate brain GABA levels (Simler et al, 1973 Loscher and Vetter, 1985) and reduced excitatory amino acids by several mechanisms on NMDA glutamate receptor (Loscher, 1993). However controversial results did exist for both glutamate and GABA (Simler et al, 1973; Anlezark et al, 1976; Loscher and Vetter, 1985; Davis, Peter, and Mctavish, 1994). The fact that GABA is a major inhibitory transmitter in the brain and that impairment of GABA-mediated inhibitions is associated with convulsions, as well as observations of a reduction in GABA neurons in both human and experimental animals caused the suffering from various types of seizures, have lead to the GABA hypothesis of epileptogenesis (Fromm, 1992; Loscher, 1993). Base on this hypothesis, admistration of VPA at therapeutic doses should lead to an increase in brain GABA levels, however it has been found that there is no real correlation between anticonvulsant action of VPA and the elevation in cerebral GABA content which was detected at very high doses (Johnston and Slater, 1982). Generally it is accepted that there are several mechanisms other than an elevation of brain GABA responsible for antiepileptic activity of VPA (Zona and Avoli, 1990).

In conclusion the present study demonstrated a potent anticonvulsant activity of Amide 1C in MES and PTZ tests, and to a lesser extent, being active in bicuculline test. A greater efficacy of Amide 1C than VPA was demonstrated in all animal models tested. Furthermore, Amide 1C was also orally active and might be rapidly absorbed from the GI tract.

Toxicity testing in terms of lethality and assessment of neurological impairment by rotorod test revealed rather favorable toxicity profile of Amide 1C. Not only the protective index (PI = TD_{50}/ED_{50}) but also the relative safety margin (LD_{50}/ED_{50}) of Amide 1C were considerably higher than those elicited by VPA. Therefore, in addition to being more potent, Amide 1C seems to offer a better therapeutic window than VPA. Moreover no depressant effect of Amide 1C and VPA was observed in locomotor activity test, while it was apparent in barbiturate sleeping times test.

With regards to possible effects of Amide 1C on cortical amino acid neurotransmitters of anesthetized rats, results from microdialysis studies demonstrated that neither inhibitory (GABA, glycine) nor excitatory (Aspartate, glutamate) amino acid level was altered by Amide 1C. Thus, it is very unlikely that an increment of inhibitory and/or a reduction of excitatory amino acid neuroransmitter could account for the anticonvulsant activity of Amide 1C.

Taken all evidence together, a newly synthesized valproic acid analogue, Amide 1C, demonstrated a potential to become an attractive anticonvulsant candidate with higher potency but rather lower toxicity than its parent compound, VPA. No major effect of Amide 1C was found on cortical amino acids of anesthetized rats. However, antagonism of $GABA_A$ receptor could partially underly anticonvulsant activity of Amide 1C in concert with other unidentified principal mechanism.

The usefulness of Amide 1C as antiepileptic drug may be hindered by the finding that its anticonvulsant activity declined rather rapidly. Further modification either chemically or pharmaceutically should be made to improve the anticonvulsant efficacy of Amide 1C.