

Chapter IV

Results

Anticonvulsant activity

Intraperitoneally given AMP has demonstrated a dose related anticonvulsant in all animal models tested except the convulsion induced by strychnine, while both NSS and PEG400 (0.1 ml/25g B.W., i.p. and 0.3 ml/25g B.W., i.p.) which were given to control groups, exhibited no protection. Similar results with lower degree of protection were observed in VPA treated animals. Optimal pretreated time for protection against electroshock of both AMP and VPA was 30 min.

1. Anticonvulsant activity against MES test

The results were expressed in terms of percentage protection against MES in mice by AMP and VPA. The ED_{50} of AMP were 17, 24, 42 mg/kg B.W. at pretreated time of 30, 60, 180 min, respectively, while corresponding values for VPA were 214, 243, 368 mg/kg B.W. (Figure 4-5)

The optimal pretreated time defined as the minimal time for the tested substance to exert its highest anticonvulsant activity was found to be 30 min for both AMP and VPA (Figure 5). Like VPA, the anticonvulsant activity of AMP was still evident at 3 hours after dosing, however, with an increment of ED_{50} (Figure 5.).

2. Anticonvulsant activity against PTZ seizure

In line with the results in MES test, AMP and VPA exhibited anticonvulsant activity against PTZ seizure in mice (Table 4). The ED_{50} was 68(53-87) and 86(62-120) mg/kg B.W. for AMP and VPA at optimal pretreated time, respectively (Figure 6).

3. Anticonvulsant activity against strychnine convulsion in mice

As shown in Table 4, the relatively high ED_{50} (> 100 mg/kg B.W.) indicated a weak protection in model tested. Neither VPA nor AMP in the dose range tested was found to be effective in strychnine-induced convulsion (Table 4).

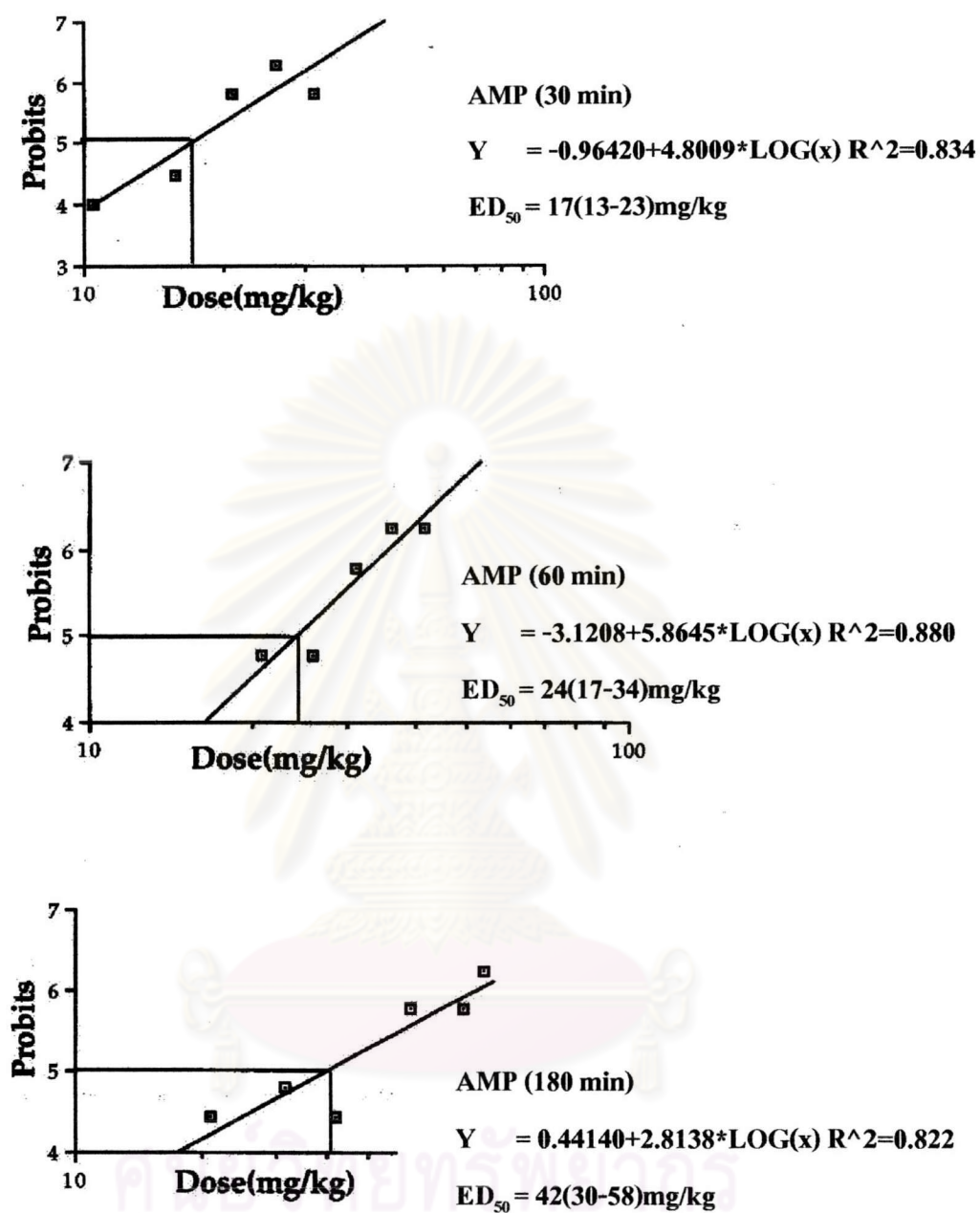


Figure 4. Log dose response curves of AMP(i.p.) on MES at 30, 60 and 180 min

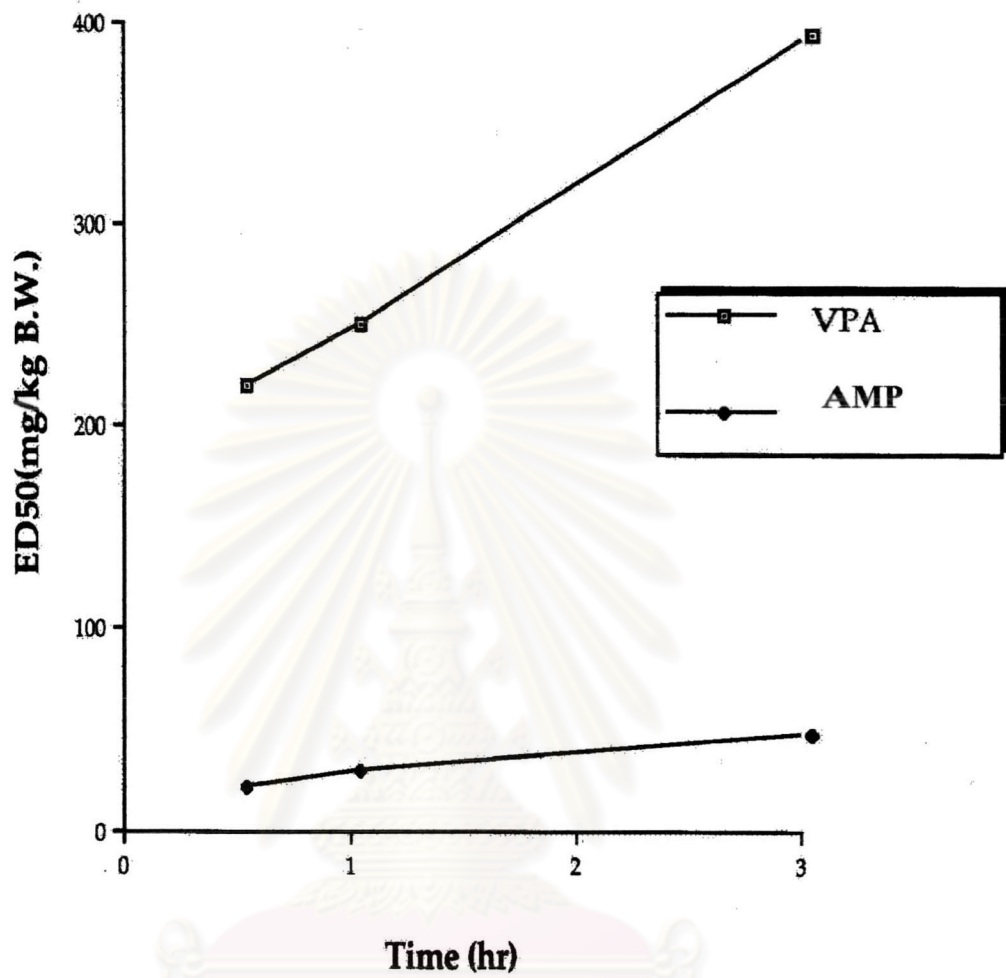
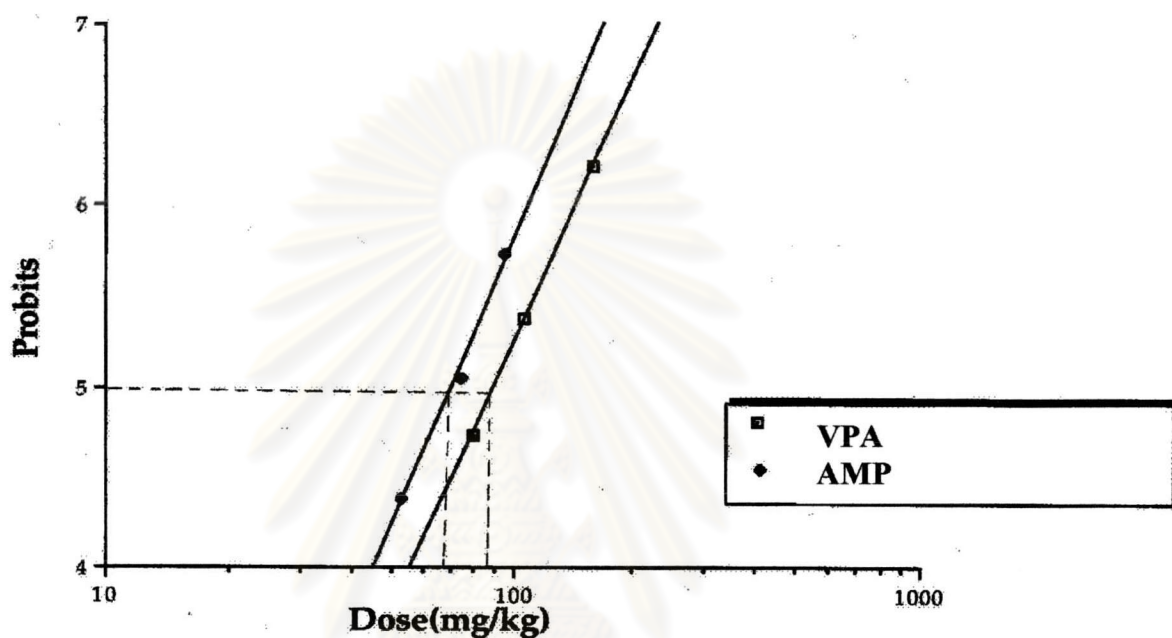


Figure 5. Protection against MES exhibited by AMP and VPA at various pretreated time in mice.



$$\text{VPA} = -4.4384 + 4.8694 \cdot \text{LOG}(x) \quad R^2 = 1.000$$

$$\text{ED}_{50} = 86(62-120) \text{mg/kg}$$

$$\text{AMP} = -4.6252 + 5.2517 \cdot \text{LOG}(x) \quad R^2 = 0.993$$

$$\text{ED}_{50} = 68(53-87) \text{mg/kg}$$

Figure 6. Log dose response curves of AMP and VPA (i.p.) on PTZ at 30 min pretreated time.

Animal model	Animals (n = 8)	ED ₅₀ (mg/kg B.W.)	
		VPA	AMP
MES test	Mice	214(199-385)	17(13-23)
PTZ seizure test	Mice	86(62-120)	68(53-87)
Strychnine-induced convulsion	Mice	>600	> 120

Table 4. Anticonvulsant activity of intraperitoneally given AMP and VPA on various animal models of epilepsy at optimal pretreated time (30 min)

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Toxicity test

1. Acute toxicity

The most frequent clinical signs observed in mice receiving high dose of AMP and VPA were ataxia, sedation, hypnosis and respiratory tract secretion. Lethality was observed within the period of 72 hours, however, most of the death occurred in 24 hours after dosing. The log dose lethality curves of AMP and VPA are shown in Figure 7. The calculated LD₅₀ of AMP and VPA was 101 and 605 mg/kg B.W., respectively. Therefore the relative safety margin (LD₅₀ / ED₅₀) of AMP in MES test was 5.94 whereas it was 2.83 for VPA. Conversely, the relative margin of safety was found to be 1.49 and 7.03 for AMP and VPA, respectively, by PTZ test

2. Neurotoxicity

At pretreated time of 30 min, AMP demonstrated a safety profile as measured by the neuroprotective index (PI = ratio of TD₅₀ obtained from rotorod test to the ED₅₀ in the MES test) of 2.24 whereas the corresponding value for VPA was 1.44 (Table 5).

Rotorod test

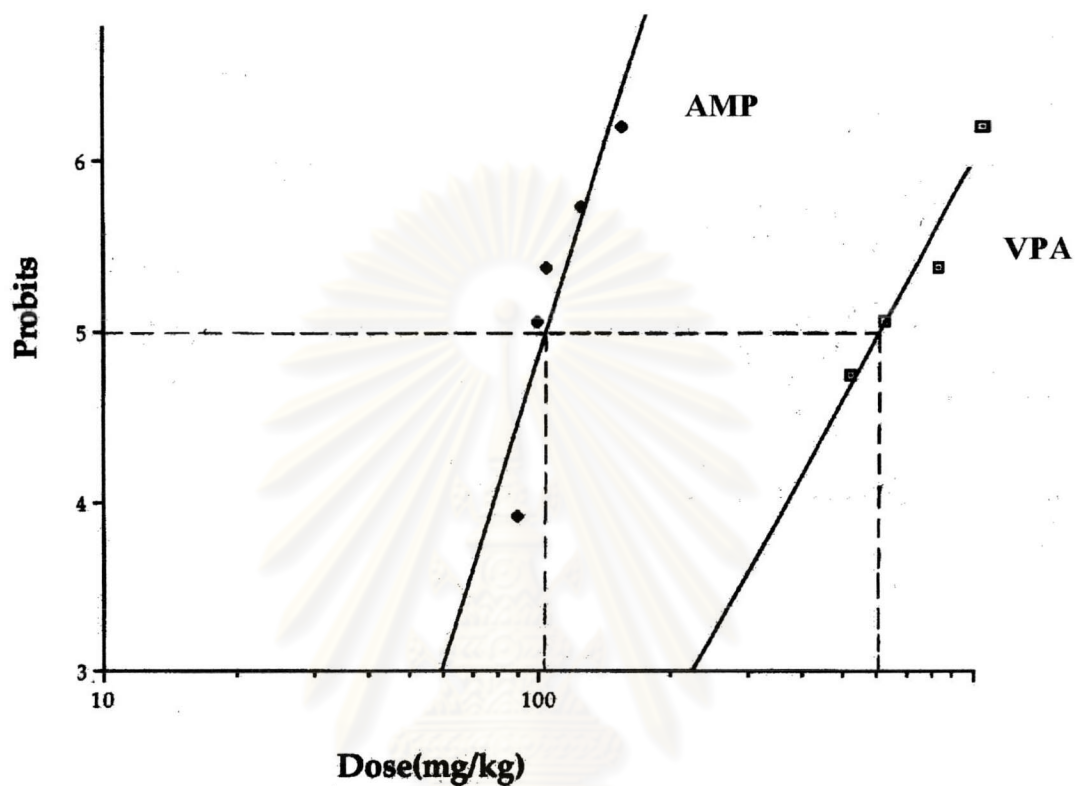
In rotorod test, control mice, receiving either NSS and PEG400, were able to maintain their equilibrium for at least 1 min on the

rotating rod in 3 successive trials. The neurological impairment as indicated by an inability of the animal to maintain their equilibrium was exhibited by an intraperitoneal administration of various doses of AMP and VPA. As illustrated in Figure 8, both AMP and VPA inhibited the rotorod performance in a dose-dependent manner. The TD_{50} of AMP and VPA at optimal pretreated time 30 min were 38 (16-91) and 309 (203-471) mg/kg B.W. respectively (Table 5).

The effect of AMP and VPA on ability of mice to perform the rotorod test were followed for 3 hours. The TD_{50} of both AMP and VPA increased as a function of time. Apparently, the TD_{50} of VPA were always higher than those of AMP at any given time (Figure 9).

Effect on barbiturate sleeping time

As shown in Figure 10, in comparison to PEG 400 AMP in the dose of 17 and 70 mg/kg B.W. significantly prolonged barbiturate sleeping time. In contrast VPA in the dose of 200 and 400 did not markedly prolong barbiturate sleeping time.



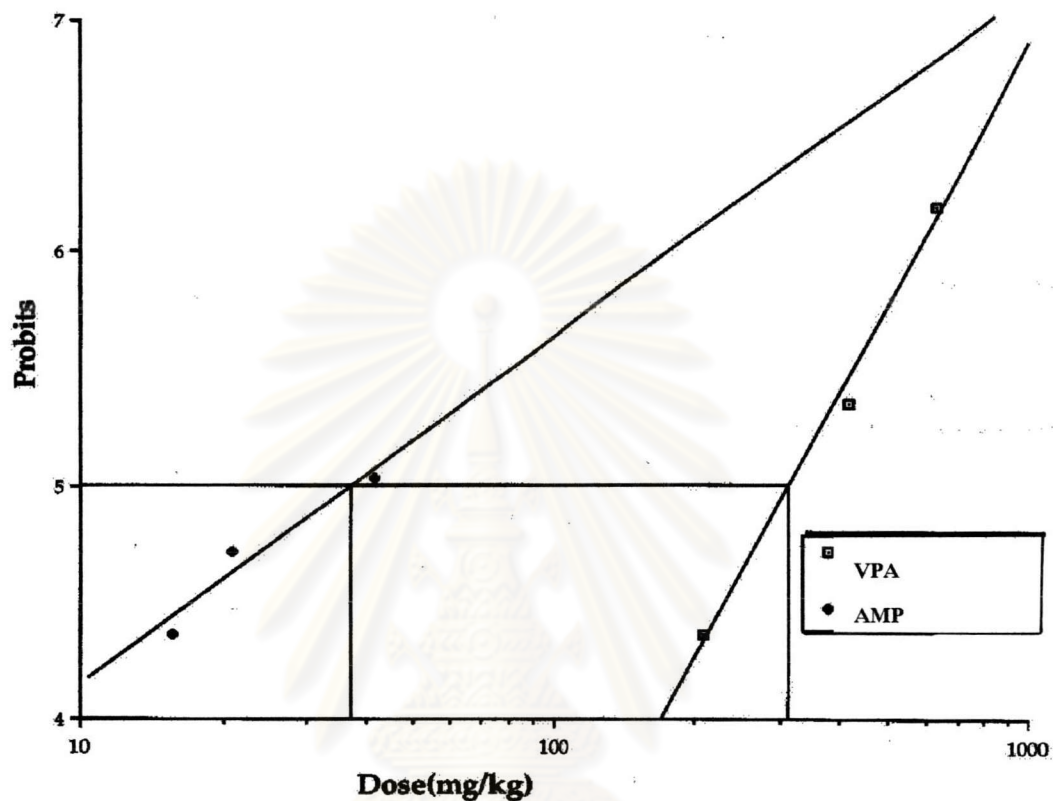
$$\text{VPA} = -7.7257 + 4.5740 \cdot \text{LOG}(x) \quad R^2=0.931$$

$$\text{LD}_{50} = 605(473-773)\text{mg/kg}$$

$$\text{AMP} = -11.332 + 8.1332 \cdot \text{LOG}(x) \quad R^2=0.825$$

$$\text{LD}_{50} = 101(88-116)\text{mg/kg}$$

Figure 7. Log dose response curves of AMP and VPA on acute toxicity (lethality) in mice.



$$\text{VPA} = -4.3848 + 3.7687 \cdot \text{LOG}(x) \quad R^2=0.990$$

$$\text{TD}_{50} = 309 \text{ mg/kg}$$

$$\text{AMP} = 2.6455 + 1.4879 \cdot \text{LOG}(x) \quad R^2=0.931$$

$$\text{TD}_{50} = 38 \text{ mg/kg}$$

Figure 8. Log dose response curves of neurotoxicity exhibited by AMP and VPA (i.p) in mice at optimal pretreated time (30 min).

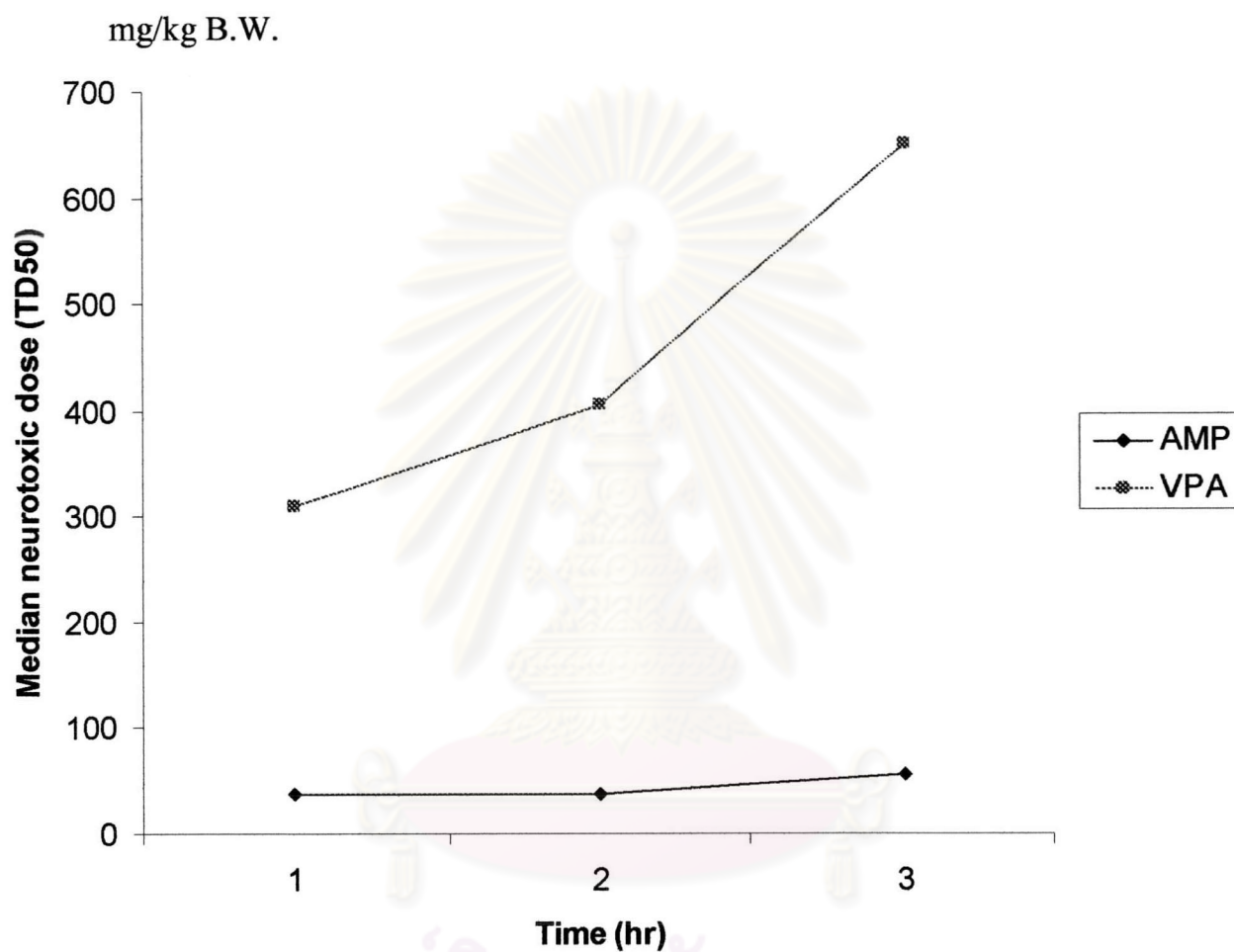
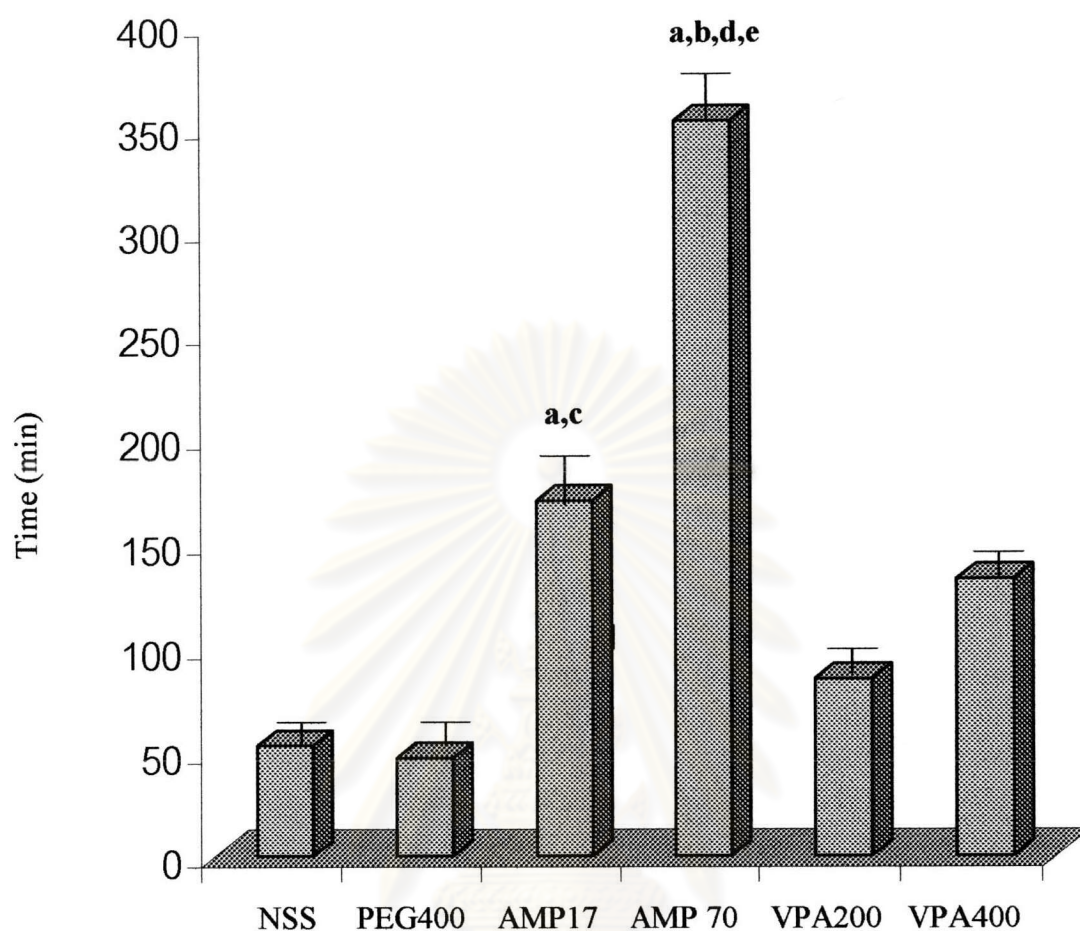


Figure 9. Neurotoxicity of AMP and VPA by rotorod test at various pretreated time in mice.

Table 5. ED_{50} , TD_{50} , LD_{50} , PI and relative safety margin of intraperitoneal administrations of AMP and VPA in MES and PTZ seizure tests in mice at optimal pretreated time (30 min).

Parameters (mg/kg B.W.)	Tests	Substances	
		VPA	AMP
ED_{50}	MES	214(199-385)	17(13-23)
	PTZ	86(62-120)	68(53-87)
TD_{50}	Rotorod	309(203-471)	38(16-91)
LD_{50}	-	605(473-773)	101(88-116)
PI(TD_{50}/ED_{50})	MES	1.44	2.24
	PTZ	3.59	0.56
Relative Safety Margin (LD_{50}/ED_{50})	MES	2.83	5.94
	PTZ	7.03	1.49



^a $p < 0.05$ denote statistically significant from PEG400

^b $p < 0.05$ denote statistically significant from AMP17

^c $p < 0.05$ denote statistically significant from AMP70

^d $p < 0.05$ denote statistically significant from VPA 200

^e $p < 0.05$ denote statistically significant from VPA 400

Figure10. Effect of intraperitoneal administration of AMP and VPA on barbiturate sleeping time (Mean \pm S.E.M., $n = 8$) in mice.

Effects on some cortical amino acid neurotransmitters levels relating to convulsion in freely moving rat.

Effects of AMP and VPA, given intraperitoneally in the dose of 70, 100, and 200, 400 mg/kg B.W. respectively, on cortical levels of aspartate, glutamate, glycine and GABA were investigated in freely moving rats by microdialysis technique. Alteration in amino acid neurotransmitter levels was expressed as a percent change from the basal value which was determined from three consecutive samples before the administration of the test substance. Qualitative and quantitative determination of the neurotransmitter in question was accomplished by HPLC (precolumn fluorescence derivatization with OPA). HPLC chromatograms of OPA-derivatized amino acids from rat cerebral cortex are shown in appendix.

In control groups, the effect of PEG400 on cortical aspartate, glutamate, glycine and GABA level was not statistically different from those of NSS in the period of 3 hours (Figures 11, 12, 13 and 14).

VPA in the dose of 400 but not 200 mg/kg B.W. significantly decreased cortical glutamate level (Figures 16 and 20) whereas both doses had no effect on either glycine or GABA (Figures 17 and 21).

A marked increase in the level of glycine and GABA were elicited exclusively by AMP in the dose of 70 mg/kg B.W. (Figures 17, 18, 21 and 22) whereas significantly decreasing level of aspartate and glutamate was

observed (Figures 15, 16, 19 and 20). Furthermore higher dose of AMP has no effect on either aspartate, or glutamate or glycine or GABA levels (Figures 19, 20, 21 and 22).



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Figure 11. Change in the rat cortical aspartate levels at various times after an intraperitoneal administration of NSS and PEG400 (n = 5)

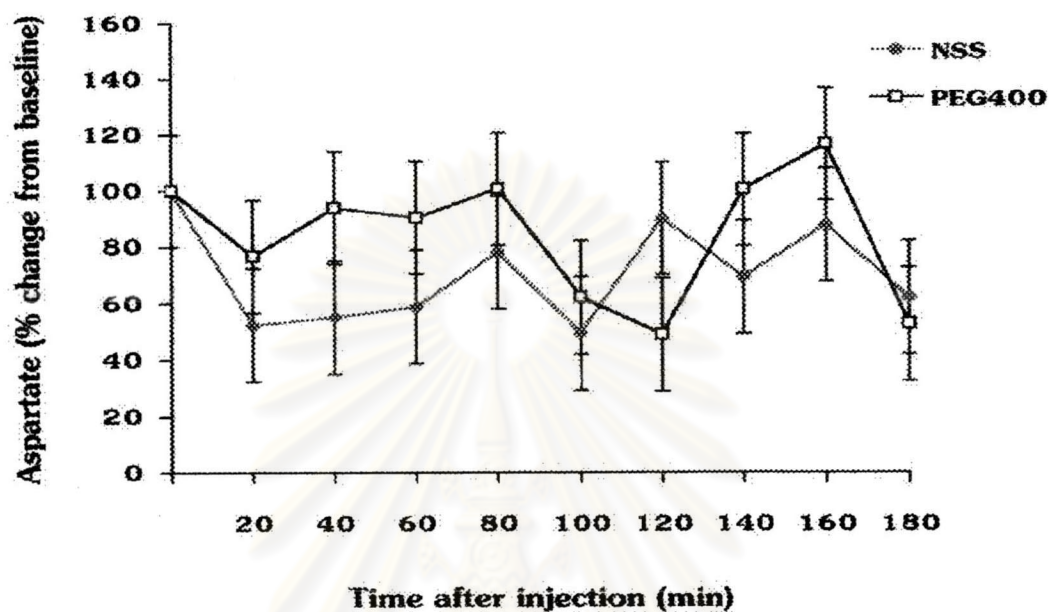


Figure 12. Change in the rat cortical glutamate levels at various times after an intraperitoneal administration of NSS and PEG400 (n = 5)

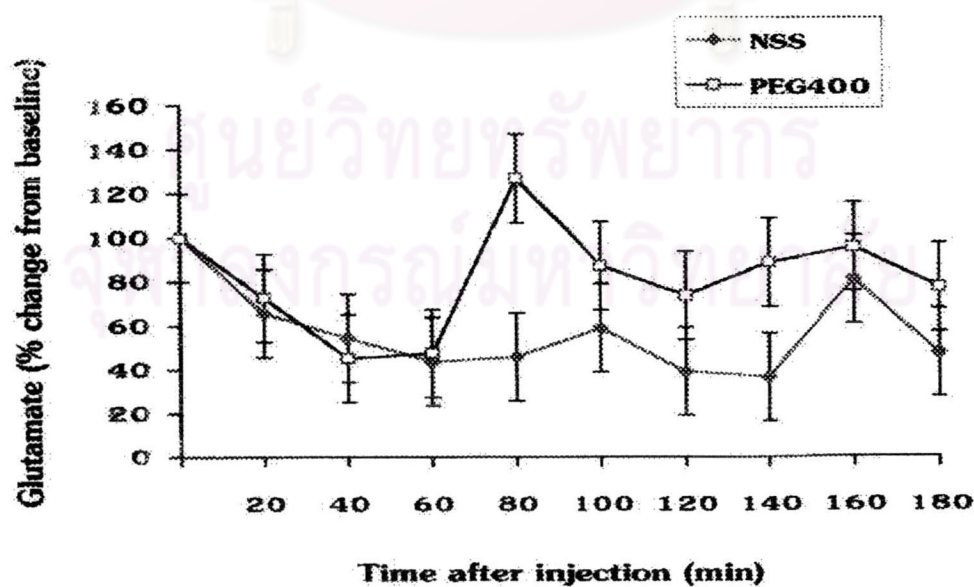


Figure 13. Change in the rat cortical glycine levels at various times after an intraperitoneal administration of NSS and PEG400 (n = 5)

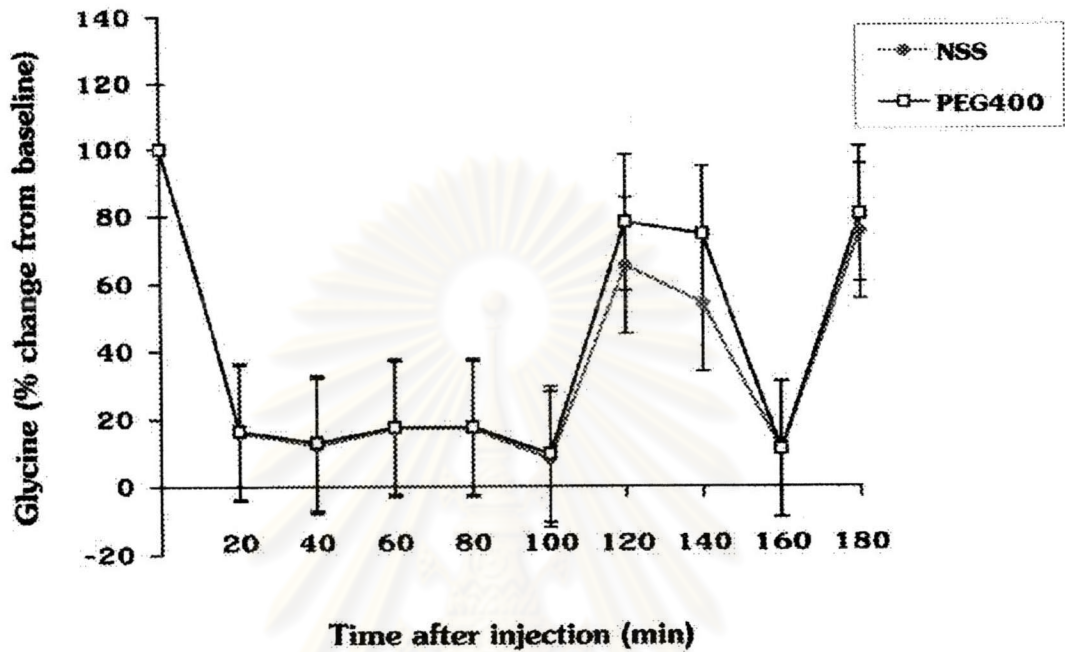
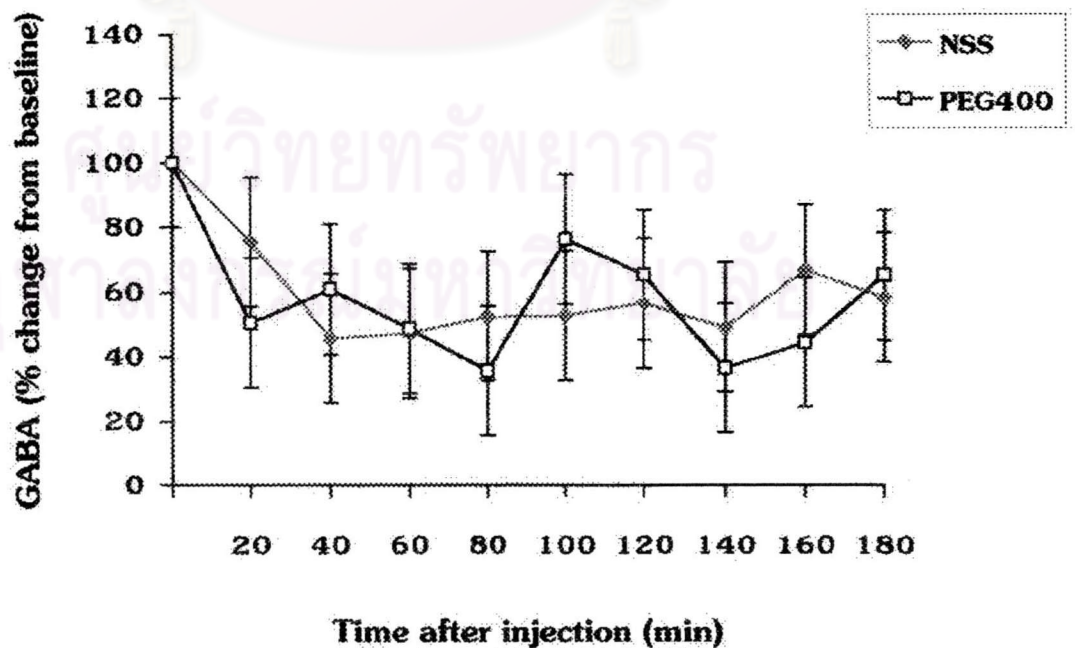
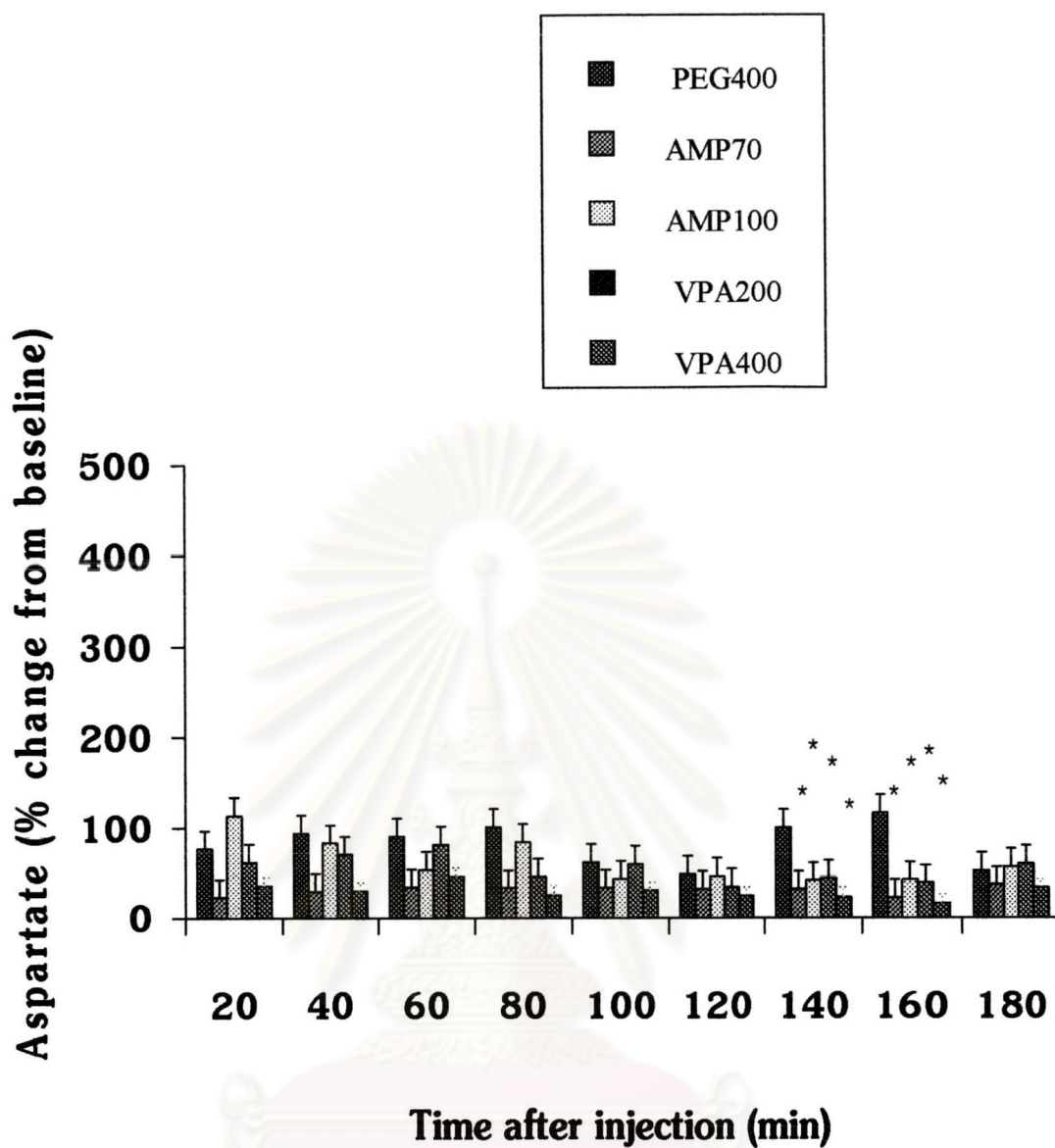


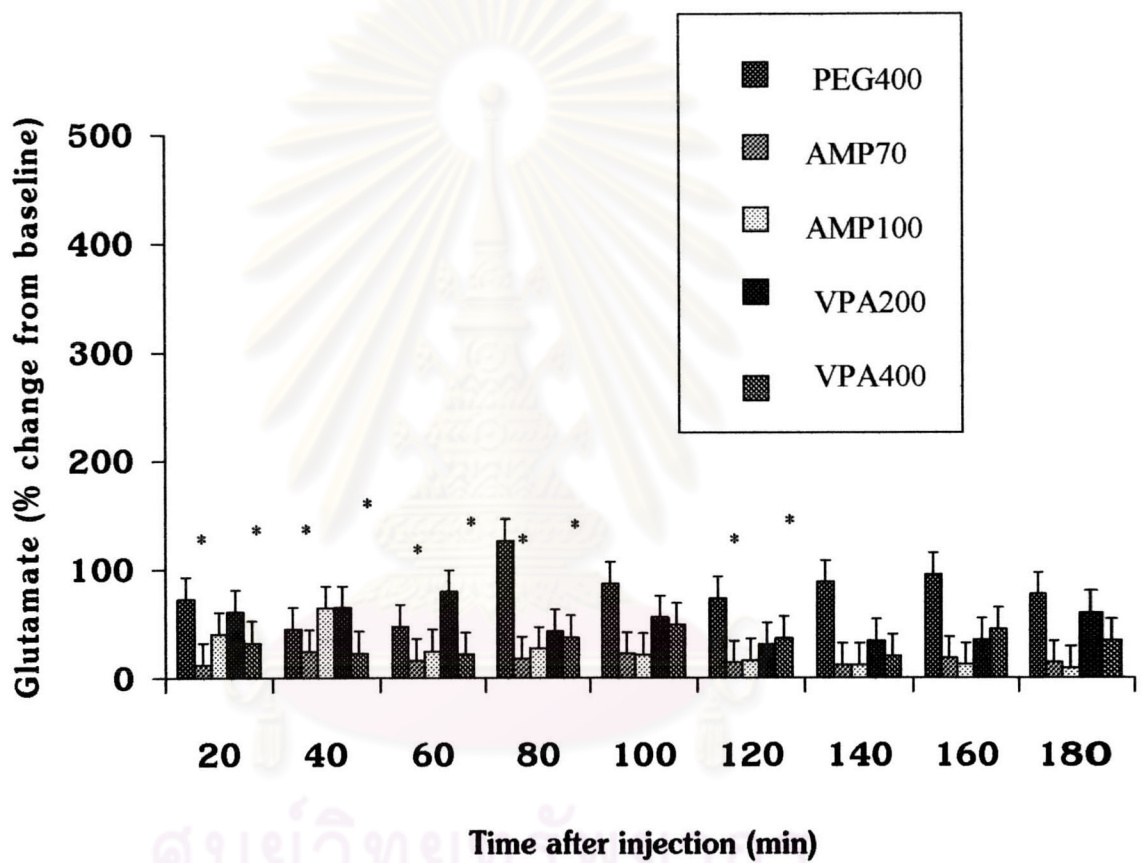
Figure 14. Change in the rat cortical GABA levels at various times after an intraperitoneal administration of NSS and PEG400 (n = 5)





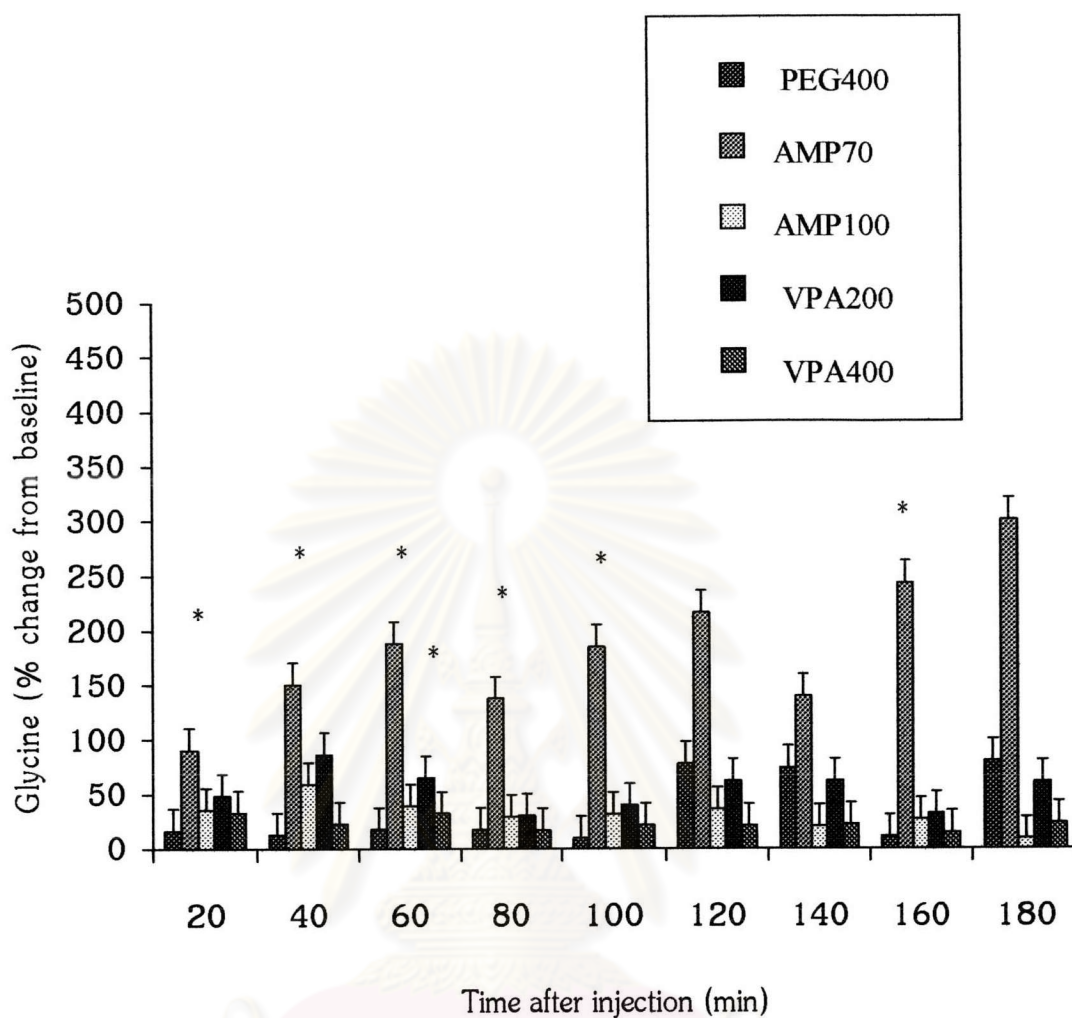
* $p < 0.05$ denotes statistically significant difference from PEG400

Figure 15. Effect of an intraperitoneal administration of AMP and VPA on the rat cortical aspartate levels at various times (n = 5)



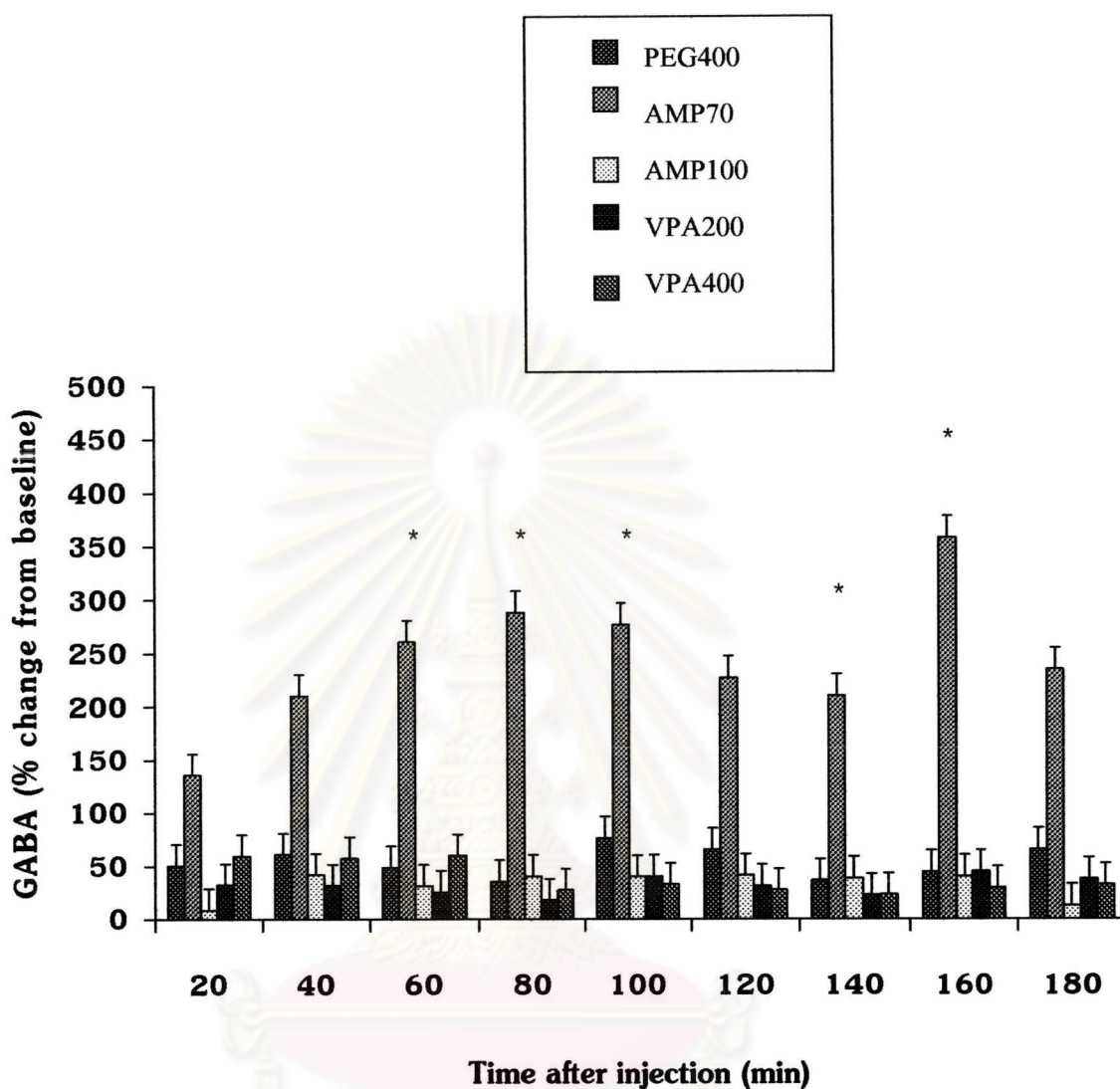
* $p < 0.05$ denotes statistically significant difference from PEG400

Figure 16. Effect of intraperitoneal administration of AMP and VPA on the rat cortical glutamate levels at various times (n = 5)



* $p < 0.05$ denotes statistically significant difference from PEG400

Figure 17. Effect of an intraperitoneal administration of AMP and VPA on the rat cortical glycine levels at various times (n = 5)



* $p < 0.05$ denotes statistically significant difference from PEG400

Figure18. Effect of an intraperitoneal administration of AMP and VPA on the rat cortical GABA levels at various times (n = 5)

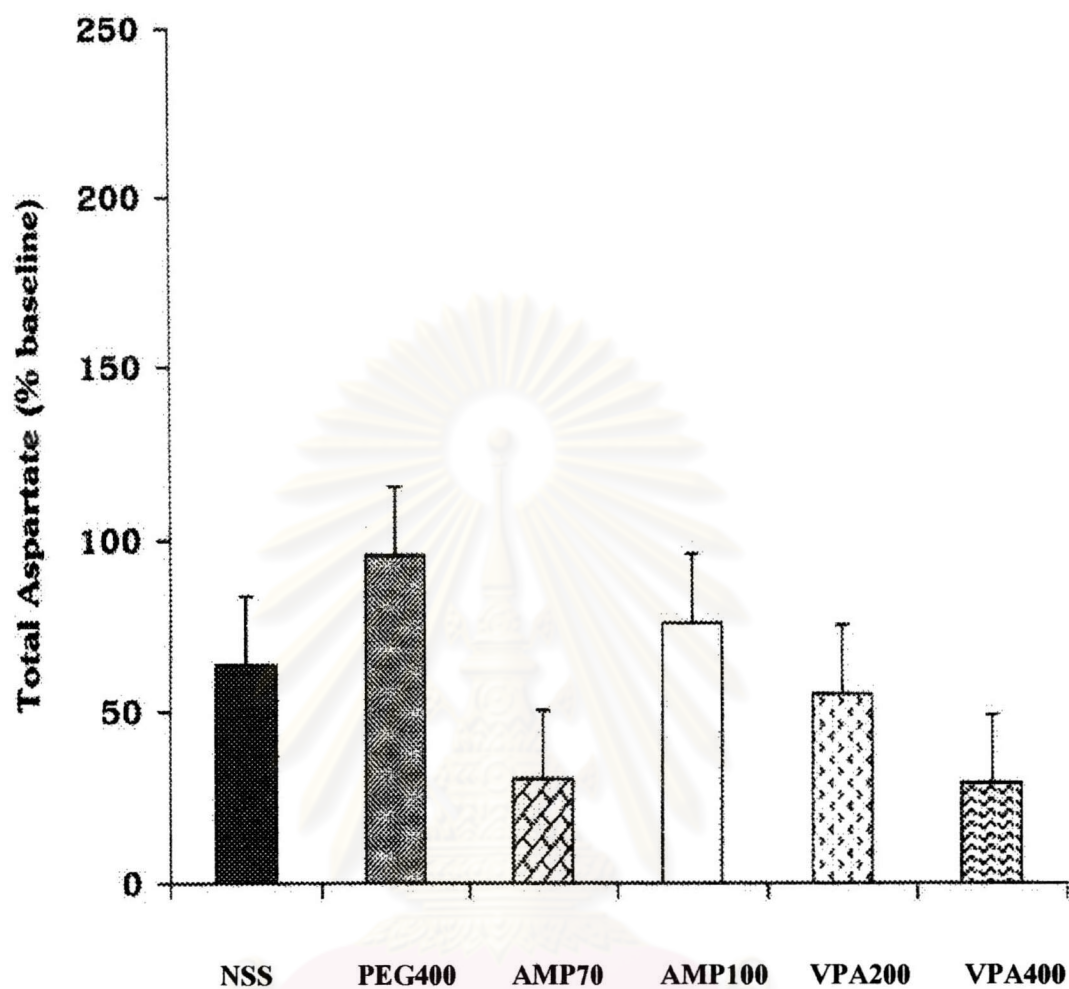
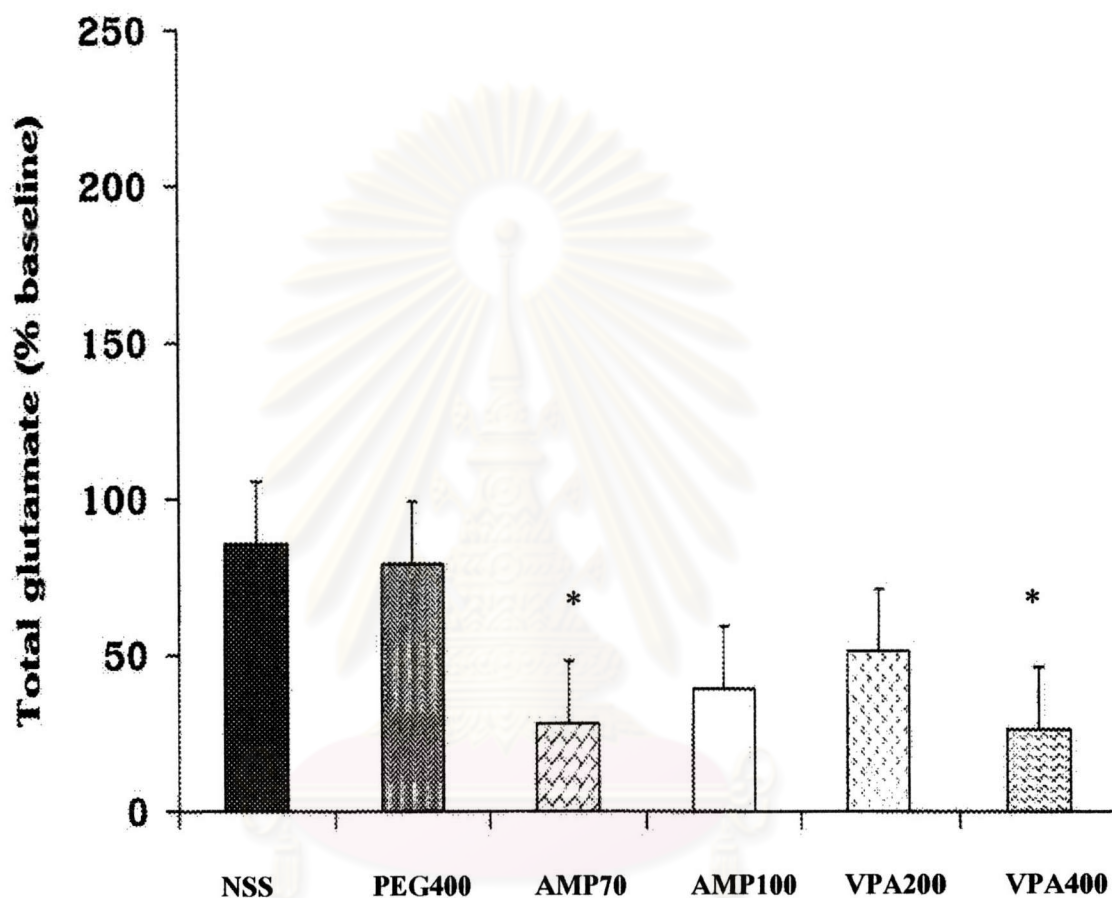
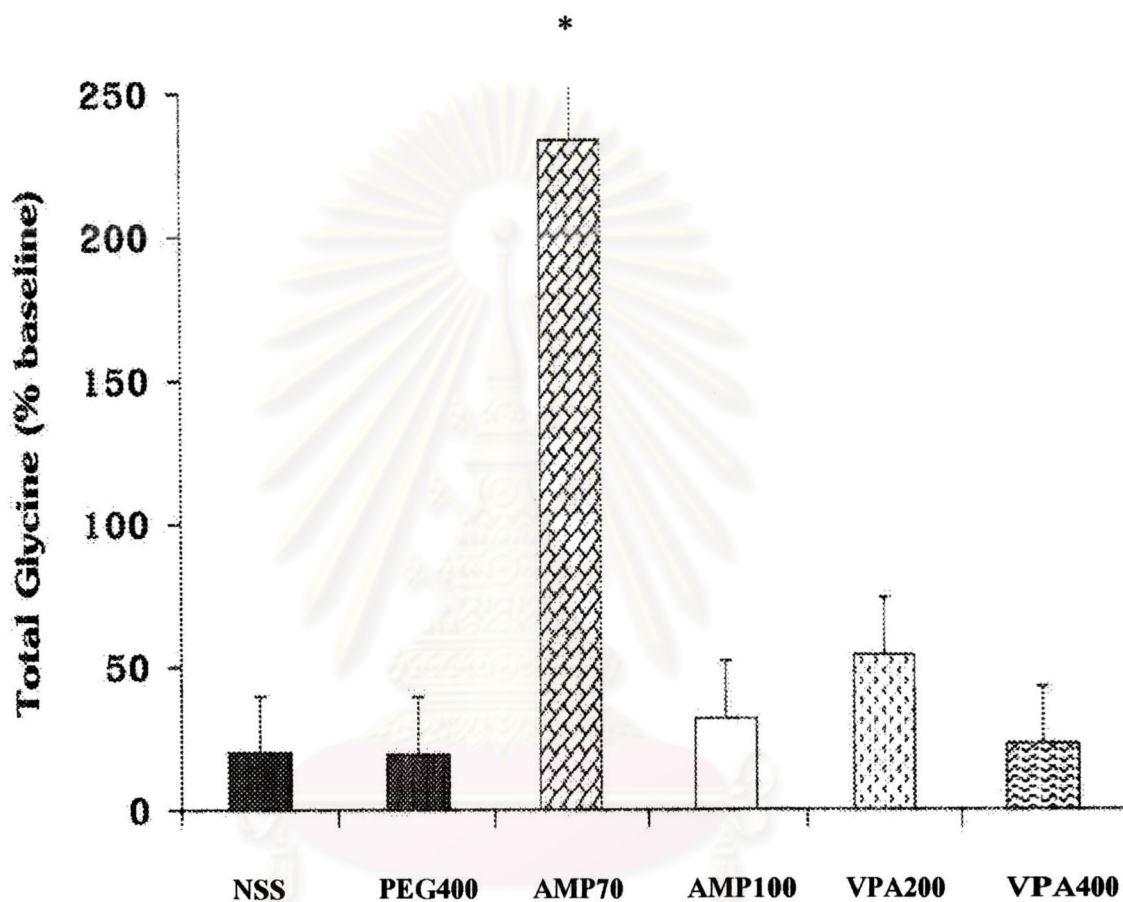


Figure19. Effect of AMP and VPA on the amount of the rat cortical aspartate in the dialysate collected for 3 hours after an intraperitoneal administration of the test substances (n = 5)



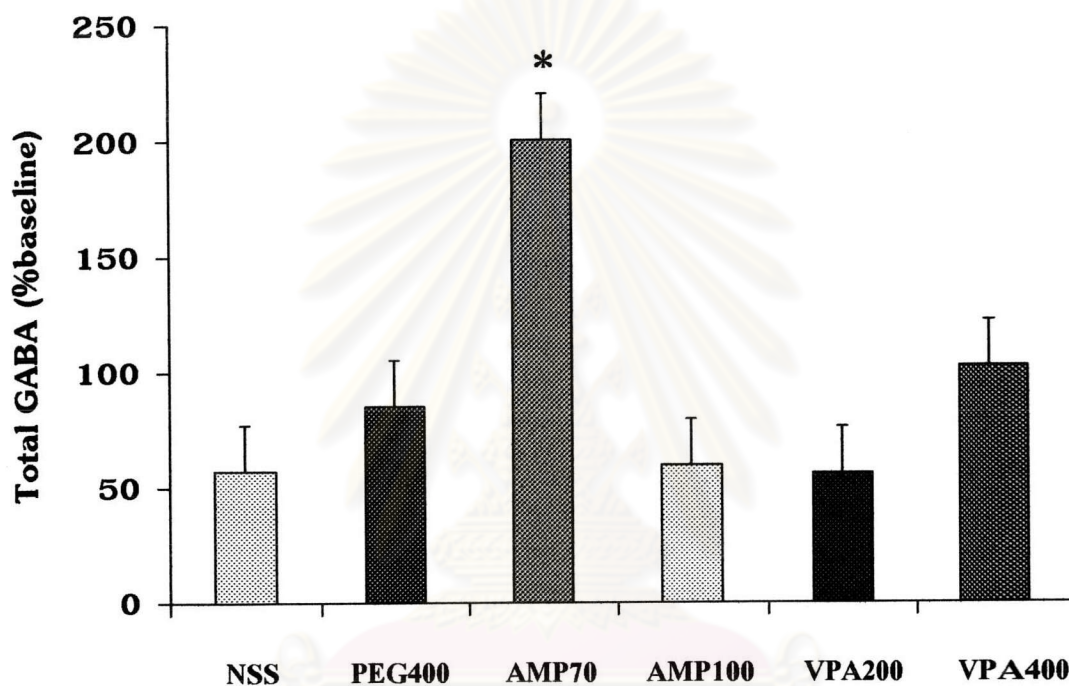
* $p < 0.05$ denotes statistically significant difference from PEG400

Figure20. Effect of AMP and VPA on the total amount of the rat cortical glutamate in the dialysate collected for 3 hours after an intraperitoneal administration of the test substances (n = 5)



* $p < 0.05$ denotes statistically significant difference from PEG400

Figure 21. Effect of AMP and VPA on the total amount of the rat cortical glycine in the dialysate collected for 3 hours after an intraperitoneal administration of the test substances (n = 5)



* $p < 0.05$ denotes statistically significant difference from PEG400

Figure22. Effect of AMP and VPA on the total amount of the rat cortical GABA in the dialysate collected for 3 hours after an intraperitoneal administration of the test substances (n = 5)