Chapter III

Results

Anticonvulsant activity

Intraperitoneal given VPU has demonstrated a dose related anticonvulsant in all animal models tested except in the convulsion induced by strychnine while PEG 400, being used as a vehicle for the tested substances, 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 VPU and VPA was 30 min (see below). Generally VPU seems to possess a higher degree of protection than VPA in both mice and rats.

1. Anticonvulsant activity against MES

The results as expressed in term of percentage protection against MES in mice of VPU and VPA given at pretreated time of 15, 30, and 60 min are summarized in Figure 5 and 6. 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 VPU and VPA. The ED_{50} of VPU and VPA at 30 min pretreated time was 66 and 242 mg/kg B.W. respectively (Table 3 and Figure 7).

Anticonvulsant activity of VPU and VPA was also demonstrated in rats (Table 3 and Figure 8) in which the ED_{50} was 67 and 233 mg/kg B.W. respectively.

2. Anticonvulsant activity against PTZ seizure

In line with the results in 1., VPU and VPA exhibited anticonvulsant activity against PTZ seizure in both mice and rats (Table 3). The ED_{50} in mice was 57 and 95 mg/kg B.W for VPU and VPA respectively (Table 3 and Figure 9). In rats, VPU demonstrated the ED_{50} of 80 mg/kg B.W. whereas the ED_{50} was 140 mg/kg B.W. for VPA (Table 3 and Figure 10).

3. Anticonvulsant activity against strychnine and bicuculline convulsion in mice

As shown in Table 3 and Figure 11, both VPU and VPA exerted anticonvulsant activity against bicuculline-induced convulsion. The relatively high ED_{50} (331 mg/kg B.W. for VPU and 393 mg/kg B.W. for VPA) indicated a weak protection in model tested. Furthermore none of the tested substances in the dose range between 100-400 mg/kg B.W. was found to be effective in strychnine-induced convulsion (Table 3).

Toxicity

1. Acute toxicity

The most frequent clinical signs observed in mice receiving high dose of VPU and VPA (500-2500 mg/kg B.W.) were ataxia, sedation, hypnosis, and respiratory tract secretion. Lethality was observed within the period of 72 hours, however, most of the death occured in 24 hours after dosing. The log dose response curves of VPU and VPA are shown in Figure 12. On the basis of the calculated LD_{50} (Table 4), VPU is less toxic than VPA (1553 vs 838 mg/kg B.W.). VPU also has the relative safety margin (LD_{50}/ED_{50}, Walker et al., 1990) in MES test and PTZ seizure higher than VPA (23.53 vs 3.46 in MES test and 27.19 vs 8.82 in PTZ seizure) (Table 4).

2. Effect on locomotor activity

Neither VPU (50 and 70 mg/kg B.W.) nor VPA (100 and 200 mg/kg B.W.) caused alteration in locomotor activity of mice (Figure 13 and 14). Though both of them tended to depress locomotor activity, no statistically significant difference between the effects of PEG 400 and tested substances was observed.

3. Effect on barbiturate sleeping time

In comparison to PEG 400, VPU and VPA in the dose of 100 and 200 mg/kg B.W. prolonged pentobarbital sleeping time. Approximately the same degree of potentiation was exhibited by lower dose of VPU and VPA (100 mg/kg B.W.) whereas in higher dose (200 mg/kg B.W.) VPU significantly demonstrated a stronger degree of depression than that of VPA (Figure 15).

Effect on some cortical amino acid neurotransmitter levels relating to convulsion in anesthetized rats

Effects of VPU and VPA, given intraperitoneally in the doses of 200 and 400 mg/kg B.W., on cortical levels of glutamate, aspartate, glycine and GABA were performed in anesthetized rats by microdialysis technique. 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 Figure 16.

With regards to PEG 400, VPA had no significant effect on the level of all neurotransmiters measured except aspartate which was significantly decreased (Table 5 and Figure 17-20). In contrast, VPU significantly decreased the levels of aspartate, glutamate, glycine and GABA in a dose related manner (Table 5 and Figure 17-20). Apparently, glycine was less sensitive to the effect of VPU as its diminution was observed only when high dose of VPU (400 mg/kg B.W.) was used (Table 5 and Figure 19).

Animal model	Animals (n=8)	ED_{50} (mg/kg B.W.)	
		VPU	VPA
MES test	mice	66 (58-75)	242 (233-251)
	rats	67 (55-81)	233 (220-247)
PTZ seizure test	mice	57 (54-60)	95 (86-105)
	rats	80 (71-90)	140 (132-148)
Bicuculline-induced convulsion	mice	331 (312-352)	393 (371-417)
Strychnine-induced convulsion	mice	> 400	> 400

Table 3 Anticonvulsant activity of intraperitoneally given VPU and VPA on various animal models of epilepsy. Numbers in parenthesis indicate range of value observed.

	Animal model	Tested substance	
	in mice	VPU	VPA
LD_{50} (mg/kg B.W.)	-	1553 (1503-1604)	838 (818-858)
ED_{50} (mg/kg B.W.)	MES test	66 (58-75)	242 (233-251)
	PTZ seizure	57 (54-60)	95 (86-105)
Relative safety margin	MES test	23.53	3.46
	PTZ seizure	27.19	8.82

Table 4 LD_{50} of VPU and VPA and relative safety margin (LD_{50}/ED_{50}) on MES test and PTZ seizure in mice. Numbers in parenthesis indicate range of value observed.

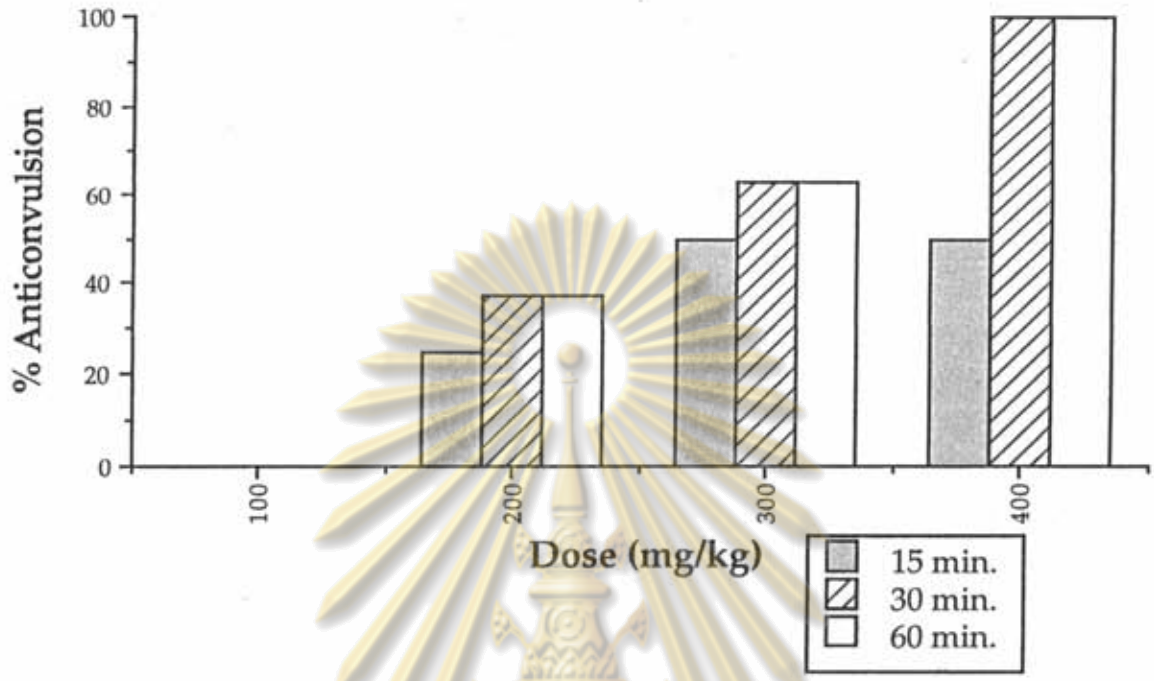


Figure 5. Effect of various pretreated time on anticonvulsant activity against MES of VPA in mice. (n=8)

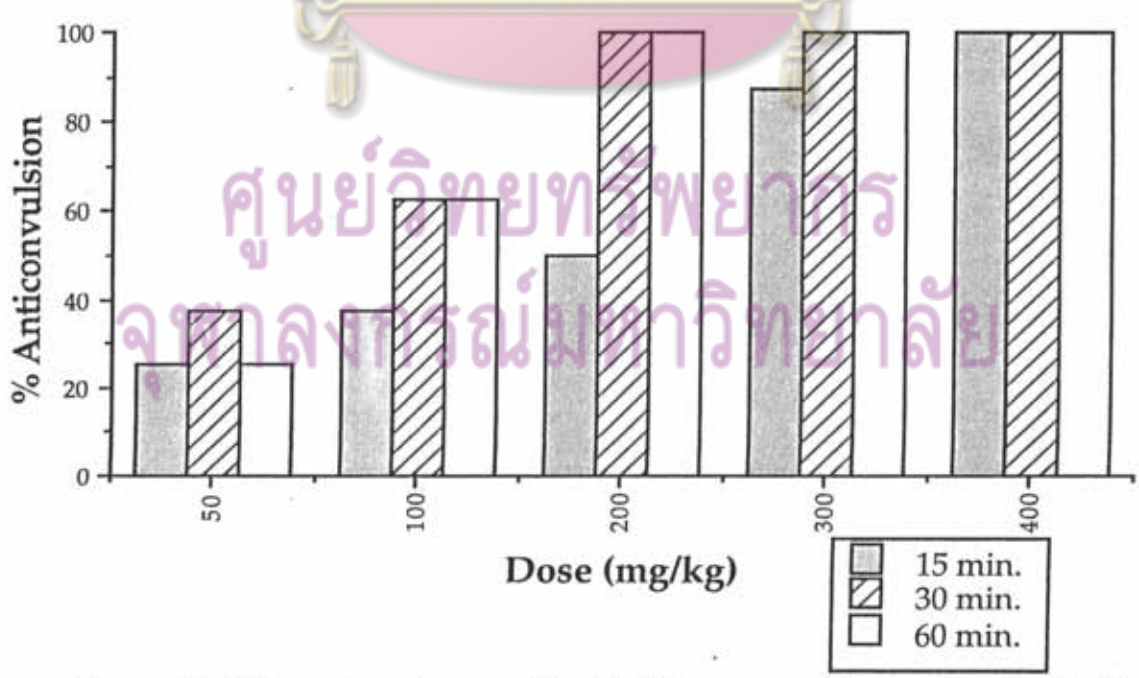
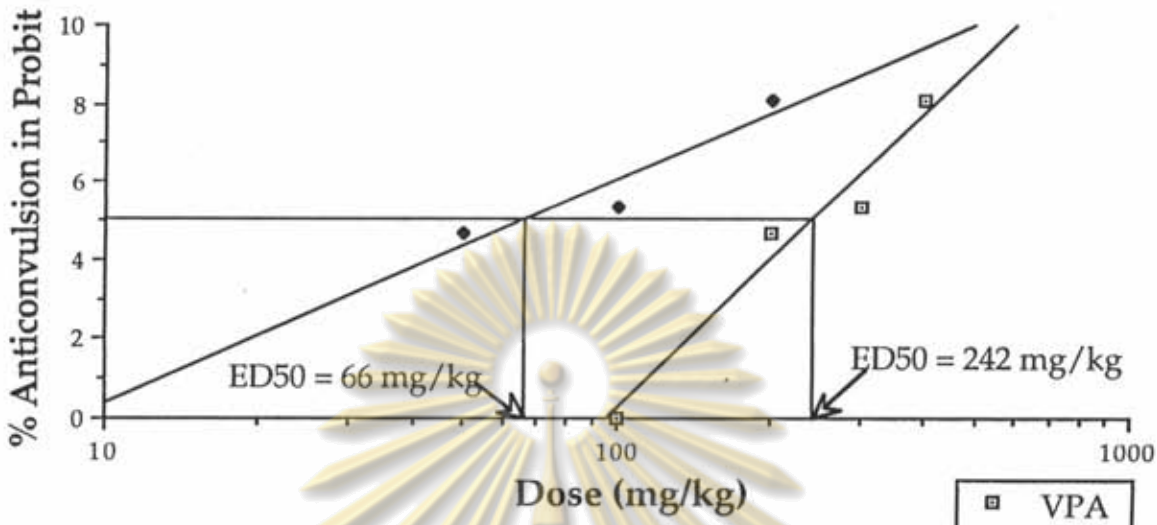


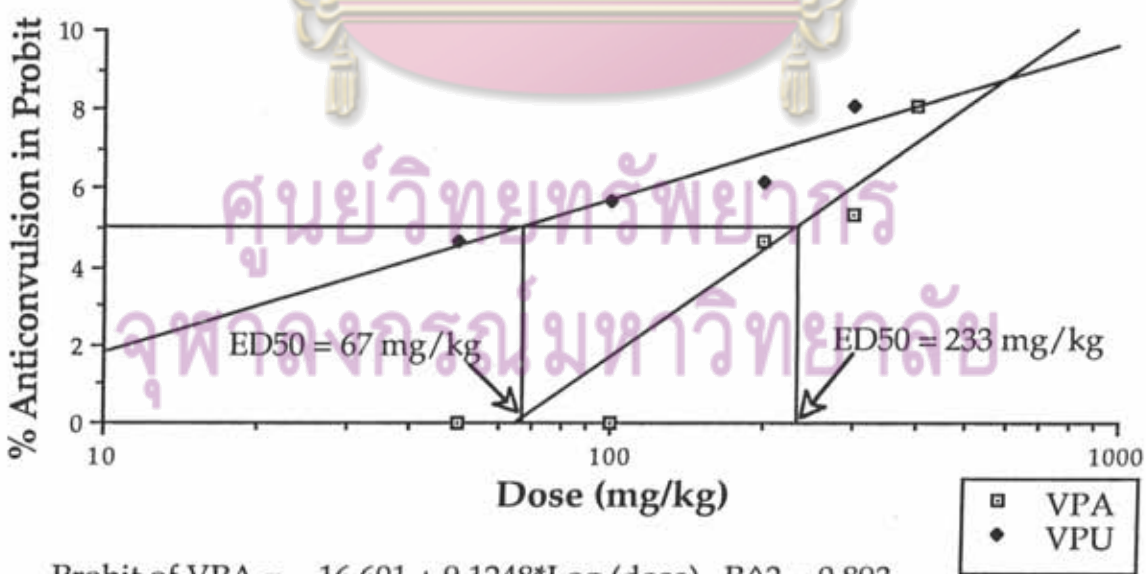
Figure 6. Effect of various pretreated time on anticonvulsant activity against MES of VPU in mice. (n=8)



Probit of VPA = - 25.013 + 12.595*Log (dose), R^2 = 0.959

Probit of VPU = - 5.2935 + 5.6617*Log (dose), R^2 = 0.884

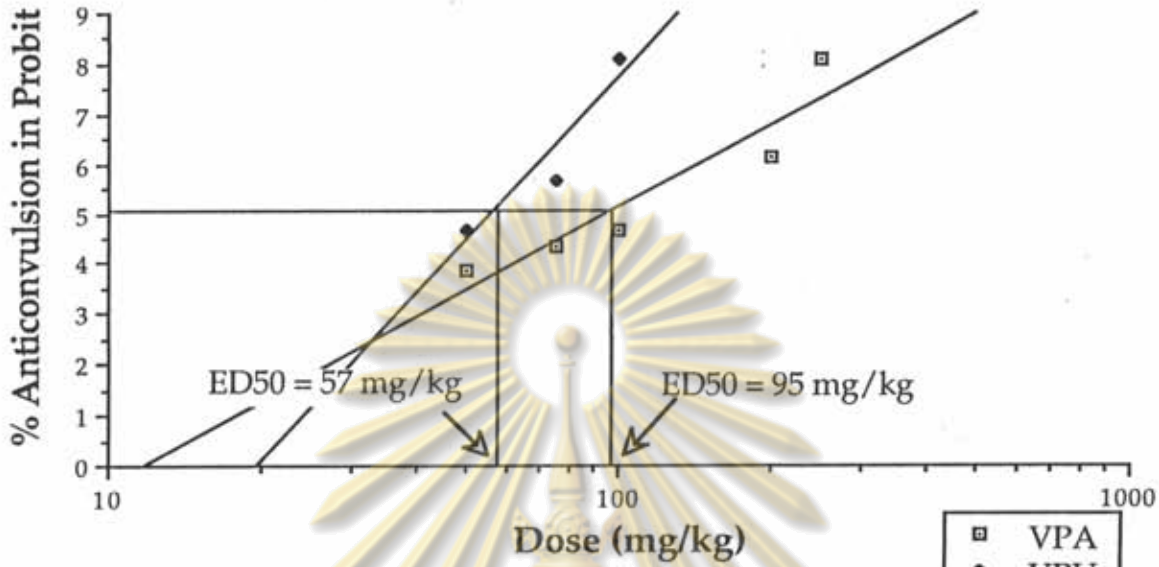
Figure 7. Log dose-response curves of VPA and VPU on MES in mice at 30 minutes pretreated time. (n=8)



Probit of VPA = - 16.601 + 9.1248*Log (dose), R^2 = 0.893

Probit of VPU = - 2.0892 + 3.8874*Log (dose), R^2 = 0.865

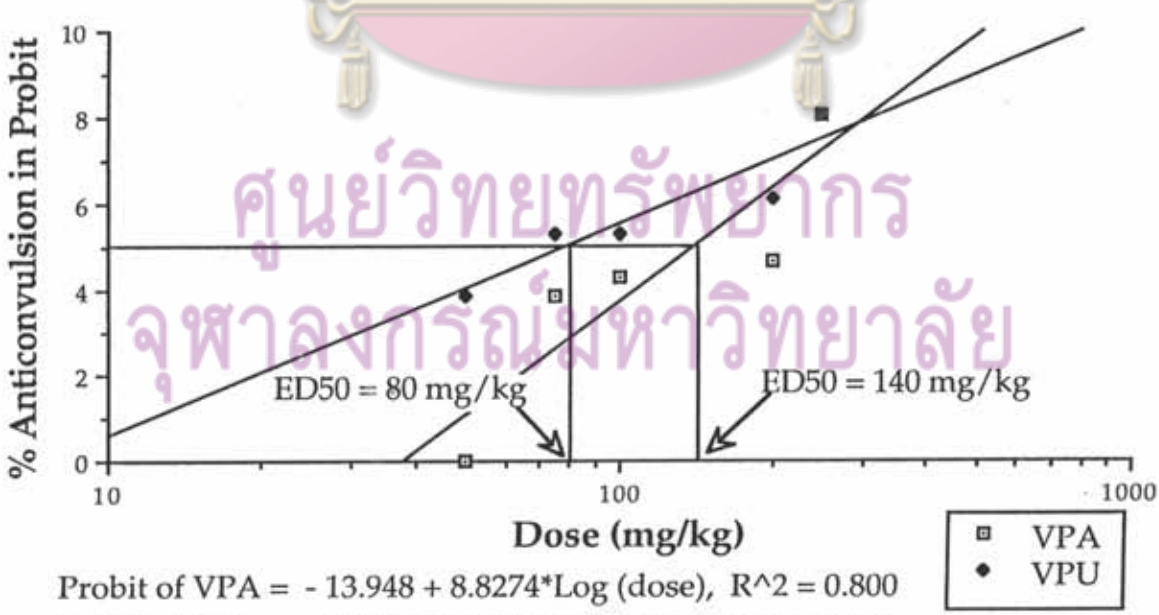
Figure 8. Log dose-response curves of VPA and VPU on MES in rat at 30 minutes pretreated time. (n=8)



Probit of VPA = - 5.9851 + 5.5522*Log (dose), R^2 = 0.886

Probit of VPU = - 14.197 + 10.950*Log (dose), R^2 = 0.892

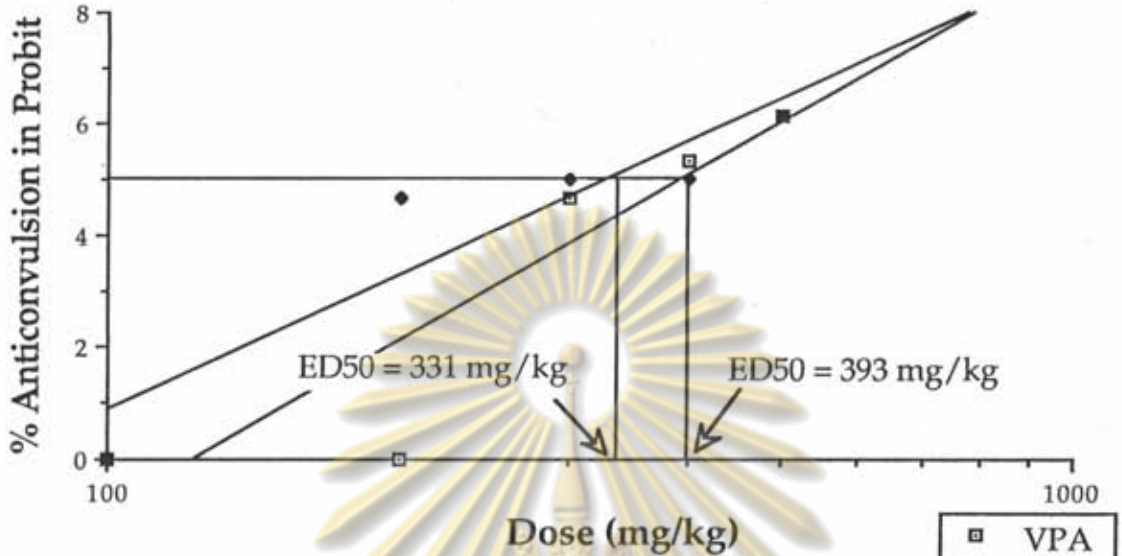
Figure 9. Log dose -response curves of VPA and VPU on PTZ seizure in mice. (n=8)



Probit of VPA = - 13.948 + 8.8274*Log (dose), R^2 = 0.800

Probit of VPU = - 4.3923 + 4.9342*Log (dose), R^2 = 0.861

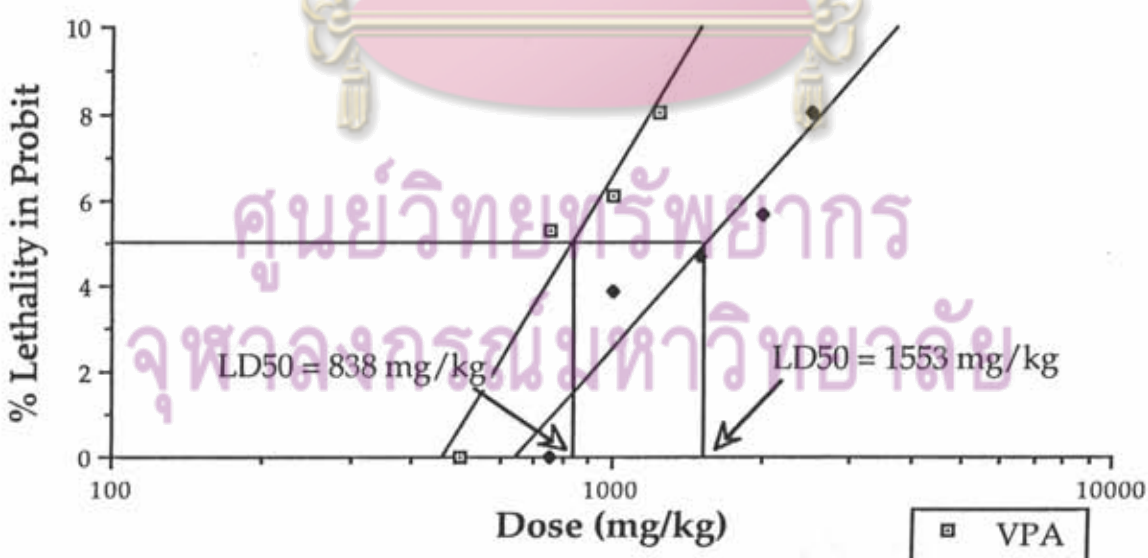
Figure 10. Log dose-response curves of VPA and VPU on PTZ seizure in rats. (n=8)



Probit of VPA = - 20.705 + 9.9076*Log(dose), R^2 = 0.834

Probit of VPU = - 15.192 + 8.0130*Log(dose), R^2 = 0.852

Figure 11. Log dose-response curves of VPA and VPU on bicuculline convulsion in mice. (n=8)



Probit of VPA = - 52.065 + 19.520*Log(dose), R^2 = 0.939

Probit of VPU = - 36.961 + 13.149*Log(dose), R^2 = 0.905

Figure 12. Log dose-response curves of VPA and VPU on acute toxicity (lethality) in mice. (n=8)

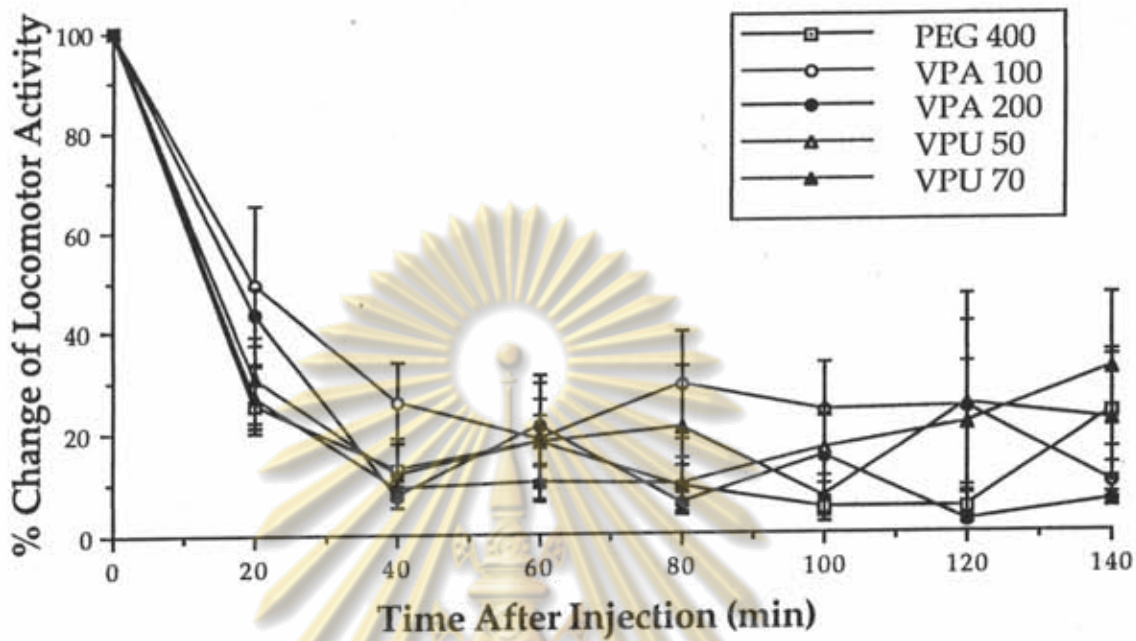


Figure 13. Effect of VPA and VPU on % change of locomotor activity in mice. (mean ± S.E.M.) (n=8)

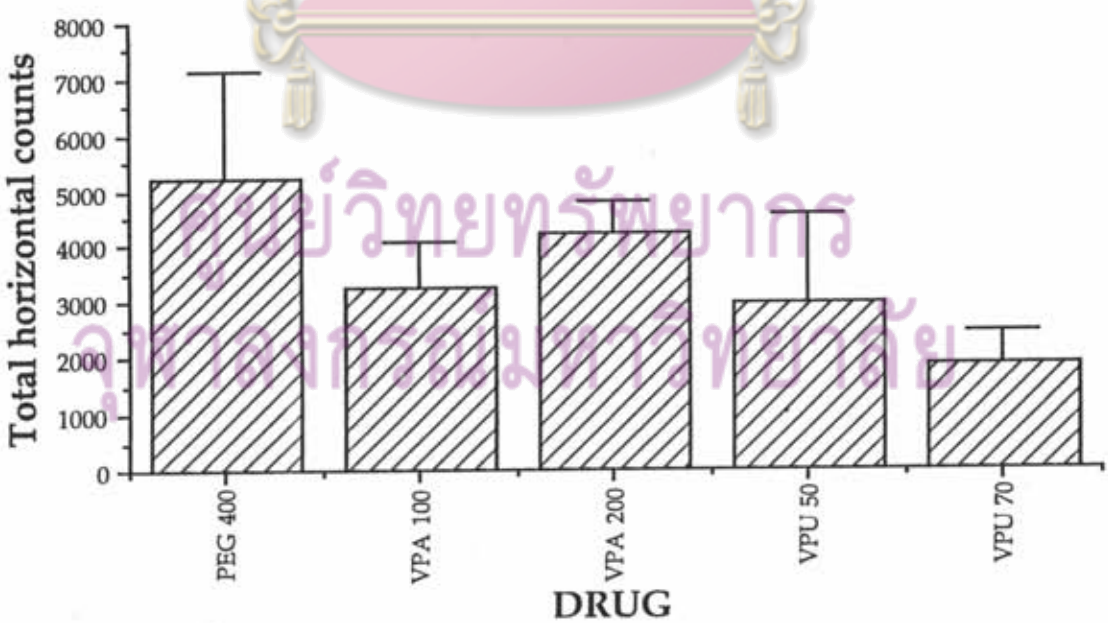


Figure 14. Effect of VPA and VPU on total horizontal counts of locomotor activity in 140 min after dosing in mice. (mean + S.E.M.) (n=8)

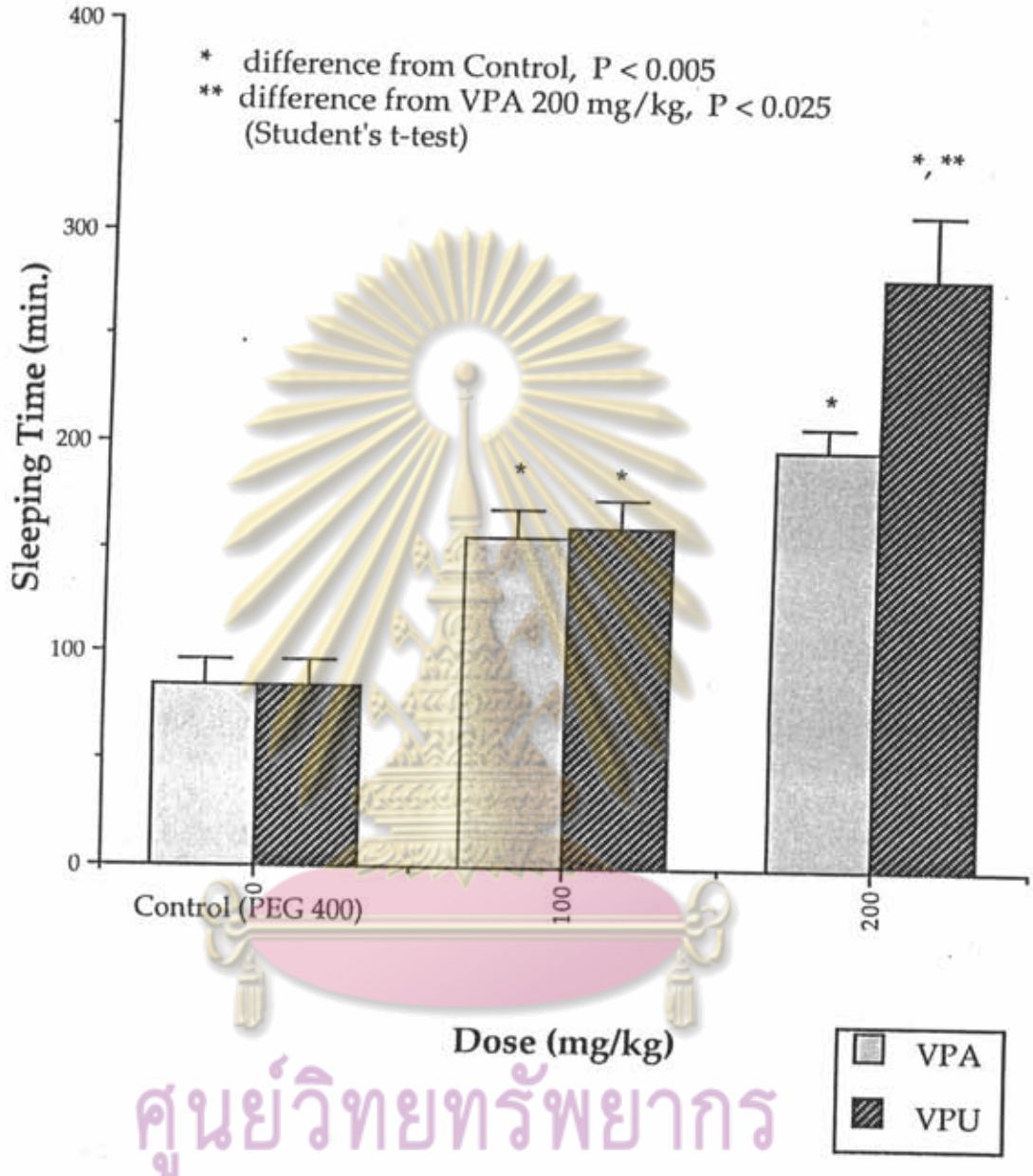


Figure 15. Effect of VPA and VPU on Pentobarbital sleeping time in mice. (mean + S.E.M.) (n=8)

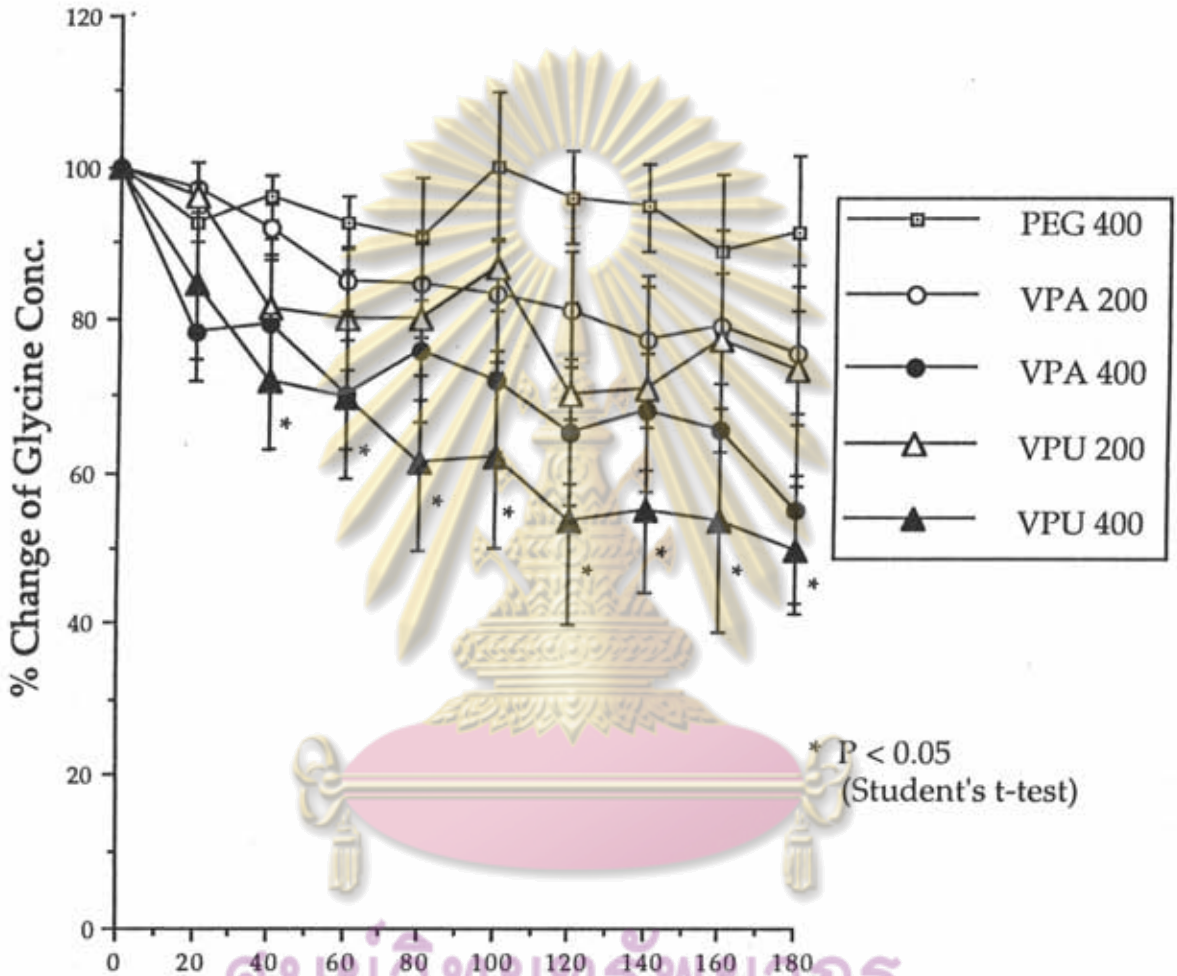
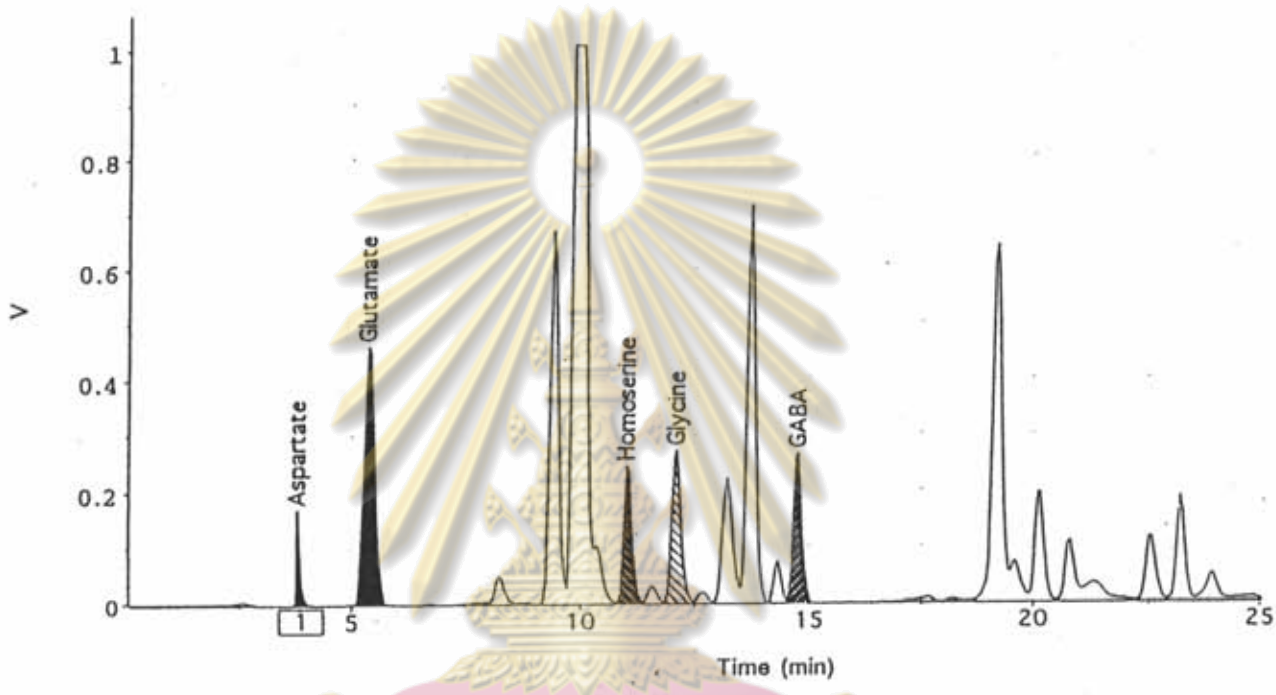


Figure 19. Effect of VPA and VPU on cortical glycine level in anesthetized rats. (mean ± S.E.M.) (n=5)



1 Control 1 (Dialys 10 + Homoser (2 uM.) 10 + OPA 50 ul.)

Peak	◇	Name	t_R (min)	Start (min)	End (min)	Area	Height (V)	Norm (%)
1		Aspartate	3.80	3.70	4.15	0.01114	0.16763	21.45
2		Glutamate	5.35	4.85	5.95	0.12560	0.45953	241.83
3		Homoserine	11.00	10.75	11.35	0.05194	0.24276	100.00
4		Glycine	12.10	11.80	12.45	0.06235	0.27036	120.04
5		GABA	14.75	14.50	15.00	0.05930	0.26400	114.17
						0.31033	1.40429	597.49

Figure 16. HPLC chromatograms of *o*-phthaldialdehyde-derivatized amino acids from rat cerebral cortex, using l-homoserine as an internal standard.

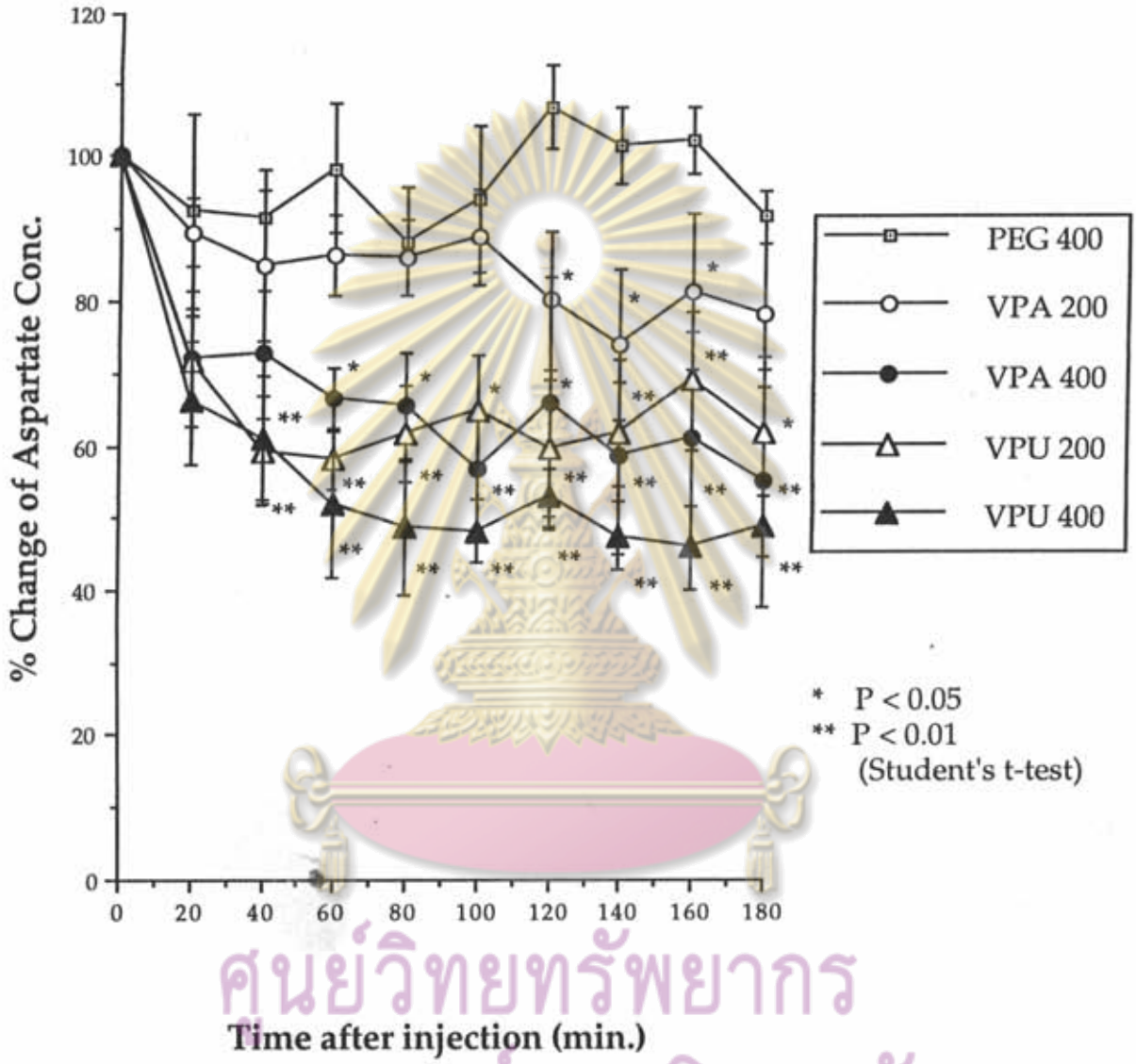


Figure 17. Effect of VPA and VPU on cortical aspartate level in anesthetized rats. (mean ± S.E.M.) (n=5)

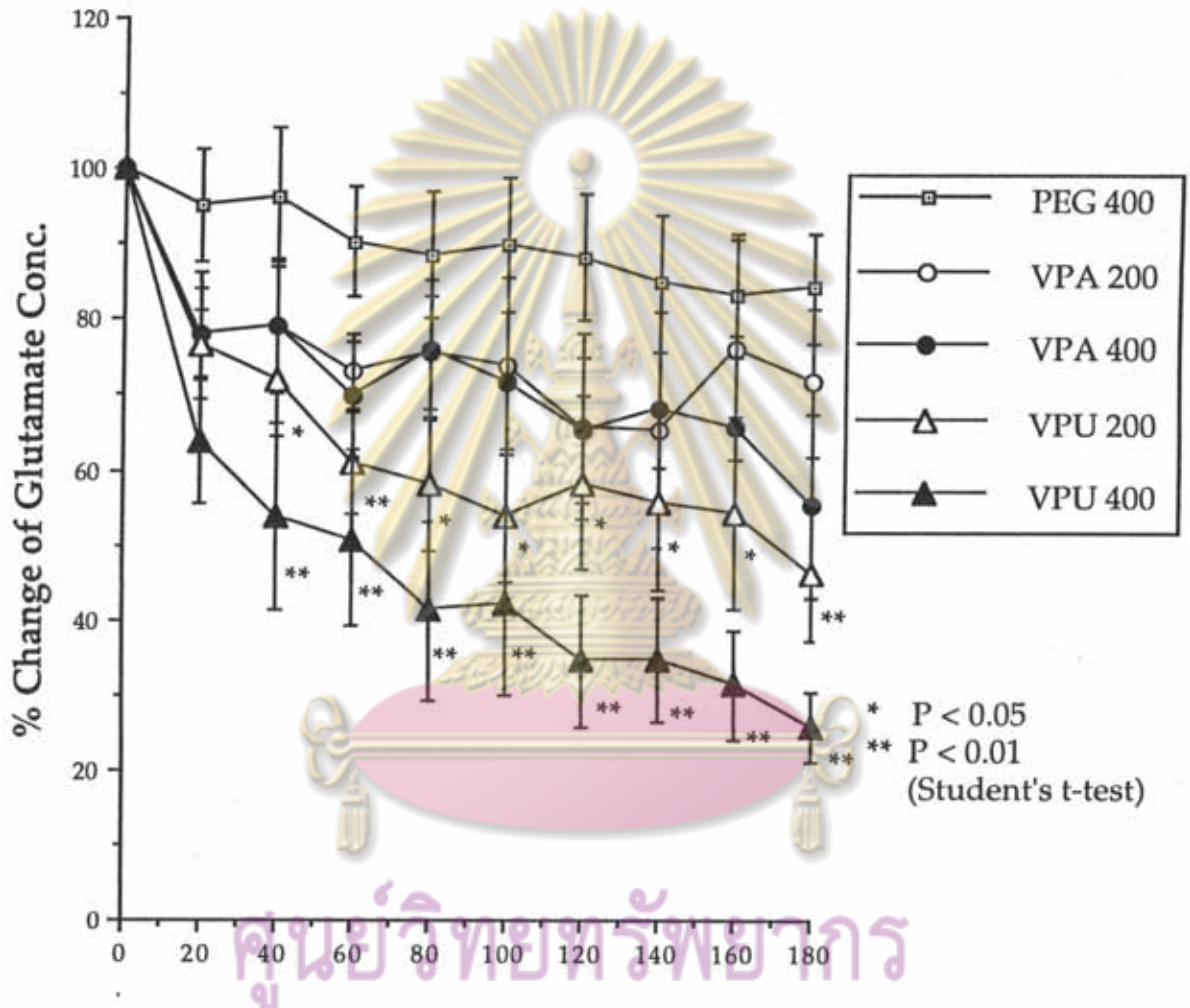


Figure 18. Effect of VPA and VPU on cortical glutamate level in anesthetized rats. (mean ± S.E.M.) (n=5)

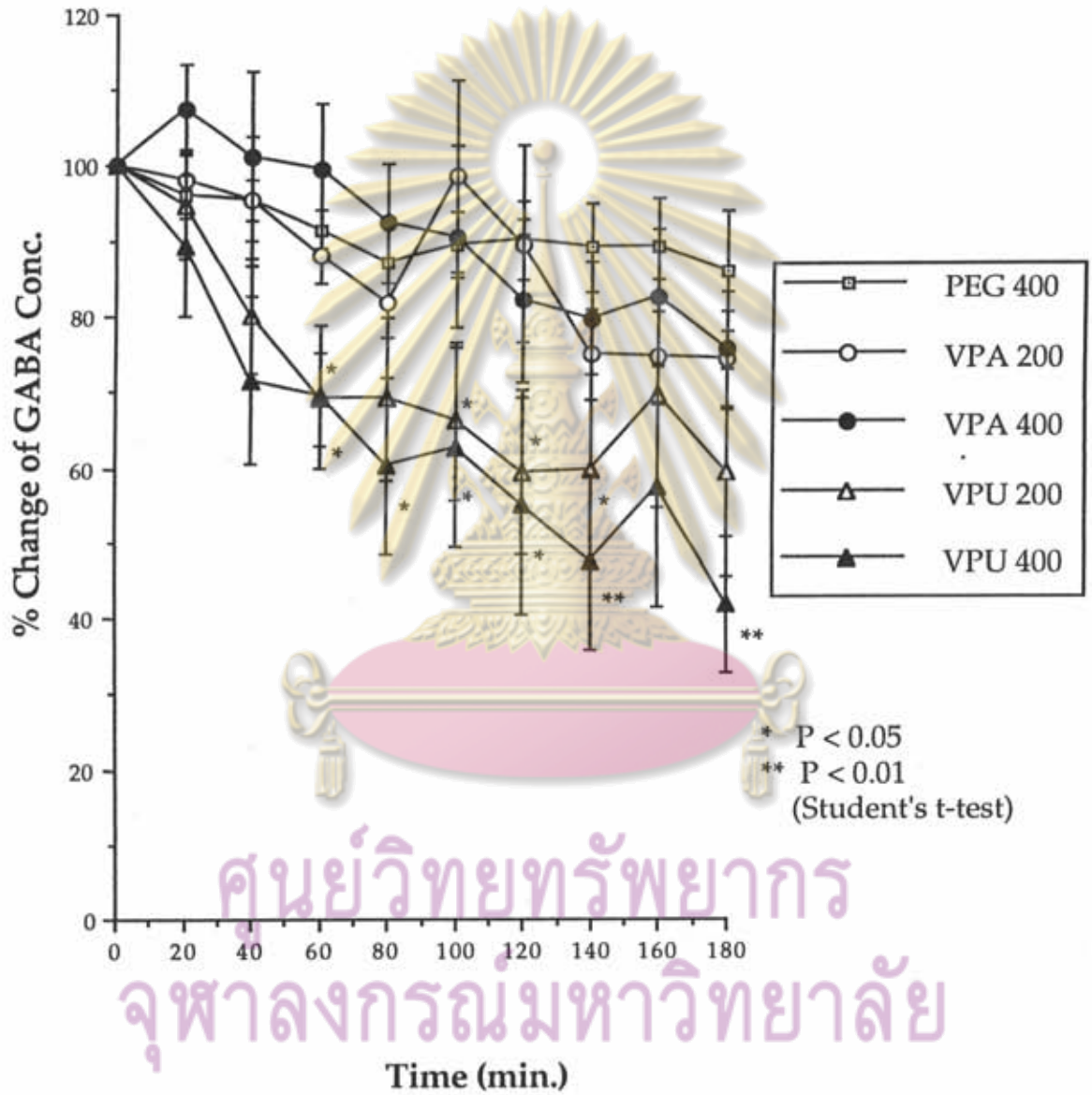


Figure 20. Effect of VPA and VPU on cortical GABA level in anesthetized rats. (mean ± S.E.M.) (n=5)

Amino acid neurotransmitters	% Decrease of rat cortical amino acid			
	VPU (mg/kg)		VPA (mg/kg)	
	200	400	200	400
Excitatory amino acids				
Aspartate	40.56**	54.26**	26.27*	45.17*
Glutamate	54.06**	74.33**	37.79^{NS}	44.94^{NS}
Inhibitory amino acids				
Glycine	29.90^{NS}	50.47*	24.83^{NS}	36.56^{NS}
GABA	40.94*	58.41**	25.88^{NS}	24.68^{NS}

Table 5. The maximal reduction effect of VPU and VPA on the levels of cortical amino acid neurotransmitters in anesthetized rats (n=5).

* $P < 0.05$

** $P < 0.01$

NS non-significance

(Compared with control (PEG 400), Student's t-test)