Chapter III

Results

1.Anticonvulsant efficacy

VPU, given either intraperitoneally or orally, demonstrated anticonvulsant activity in MES model. While both NSS and PEG400 (0.1 ml/25g B.W. i.p. and 0.3 ml/25g B.W. p.o.), which were given to control groups, exhibited no protection. In comparison, VPU clearly demonstrated 2-3 times greater potency than VPA in protection against MES. Like VPA, the anticonvulsant activity of VPU was still evident at 12 hours after dosing, however, with an increment of ED_{50} (Figure 10).

1.1 Anticonvulsant activity against MES

As shown in Figure 9, intraperitoneally given VPU and VPA demonstrated a protection against MES in a dose dependent manner exhibiting the ED_{50}, at pretreated time of 30 min, of 71 and 199 mg/kg B.W. respectively.

VPU was also orally active exhibiting the ED_{50} which were about 3-6 times higher than the ED_{50} of the intraperitoneal route (Figure 11). Among the pretreated time of 30, 60 and 120 min, the lowest oral ED_{50} (215 mg/kg B.W.) was noted at the pretreated time of 120 min (Figure 13).

Similar results were exhibited by VPA as well. The ED_{50} of VPA when given orally were 1341, 512 and 522 mg/kg B.W. at pretreated time of 30, 60 and 120 min respectively (Figure 12), giving the proportion of approximately 3-7 times to its ED_{50} by the intraperitoneal route at different times.

1.2 Duration of anticonvulsant activity

In MES test, the ED_{50} of intraperitoneally given VPA and VPU were determined for 12 hours after dosing. The ED_{50} of both VPU and VPA increased as a function of time demonstrating the final ED_{50} of 431 and 565 mg/kg B.W. at 12 hours against the ED_{50} of 75 and 216 mg/kg B.W. in the first hour respectively (Figure 10; Table 2). Though the ED_{50} of VPU were always lower than those of VPA at any given time, the diparity was profoundly distinct at time between 1-3 hours (Figure 10).

2. Neurotoxicity

At pretreated time of 30 min, VPU demonstrated a safety profile as measured by the neuroprotective index (PI = ratio of TD_{50} from rotorod to the ED_{50} in the MES) of 8.8 whereas the corresponding value for VPA was 1.3. Further more, a relatively minor inhibition of rotorod performance was exhibited by VPU at the dose 3-5 times higher than its ED_{50} in protection against MES and this finding hold true throughout the observation period of 12 hours (Figure 15).

2.1 Impairment of neurologic function

In rotorod test, a control mice, receiving either NSS or PEG400, were able to maintain their equilibrium for at least 1 min on the rotating rod in 3 successive trials. Neurological impairment as indicated by an inability of the animals to maintain their equilibrium was exhibited by an i.p. administration of various doses of VPU and VPA. Apparently,VPU demonstrated a lower neurotoxicity than those of VPA. The TD_{50} at 30 min pretreated time of intraperitoneally given VPU and VPA were 625 and 265 mg/kg B.W. respectively (Figure 14). These resulted in a higher neuroprotective index of VPU than that of VPA. As shown in Table 3, the protective indices were 8.8 and 1.3 for VPU and VPA respectively.

2.2 Duration of neurotoxicity

The effects of VPU and VPA on ability of mice to perform the rotorod test were followed for 12 hours. The results, expressed as percentage of falling mice at various times (1/2 - 12 hours), were illustrated in Figure15. At the dose equivalent to the ED_{50} of VPA but 3-5 times higher than its own ED_{50} in the MES test, VPU (300 and 400 mg/kg B.W. i.p.) demonstrated a minor degree of neurotoxicity (0-20 percent) throughout the observation peroid. A stronger inhibition of rotorod performance (30-90 percent) was elicited by VPA (300 and 400 mg/kg B.W. i.p.).

3. *In vitro* degradation of VPU by liver and brain homogenate.

Degradation of VPU to VPA was not demonstrable by an incubation of VPU with either brain or liver homogenates. No statistically significant difference in VPA level between control and treated group was observed in either brain or liver homogenate preparation (Figure 17 and 18).

4. Effects on some cortical amino acid neurotransmitters relating to convulsion in awake 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. The basal levels of amino acids expressed as nmol/10μl dialysate, were 0.062 ± 0.008 for aspartate (n=29), 6.080 ± 0.739 for glutamate (n=29), 0.071 ± 0.068 for glycine, (n=30), and 1.490 ± 0.231 for GABA (n=30).

In control groups, the effect of PEG400 on the spontaneous release of aspartate, glutamate, glycine and GABA was not statistically different from those demonstrated by NSS (Figure 20, 21, 22 and 23).

As illustrated in Figure 28, neither VPU nor VPA exhibited significant effect on the level of aspartate throughout the observation peroid.

VPA in the dose of 200 but not 400 mg/kg B.W. significantly decreased cortical glutamate level (Figure 29). A marked increase in the level of glycine was

elicited exclusively by VPA in the dose of 400 mg/kg B.W. (Figure 30). Almost 2 folds increase in GABA level was demonstrated by VPA in which a slightly stronger effect seemed to be produced by the higher dose (400 mg/kg B.W.). However, this was not statistically different from the effect of VPA 200 mg/kg B.W. (Figure 31).

In comparison to VPA, similar effect of VPU was observed on the level of glutamate in which VPU in the dose of 200 mg/kg B.W. significantly decreased glutamate level whereas no significant effect was elicited by VPU in the dose of 400 mg/kg B.W.(Figure 29). On the contrary, VPU did not exerted any major effect on either glycine or GABA level (Figure 30 and 31).

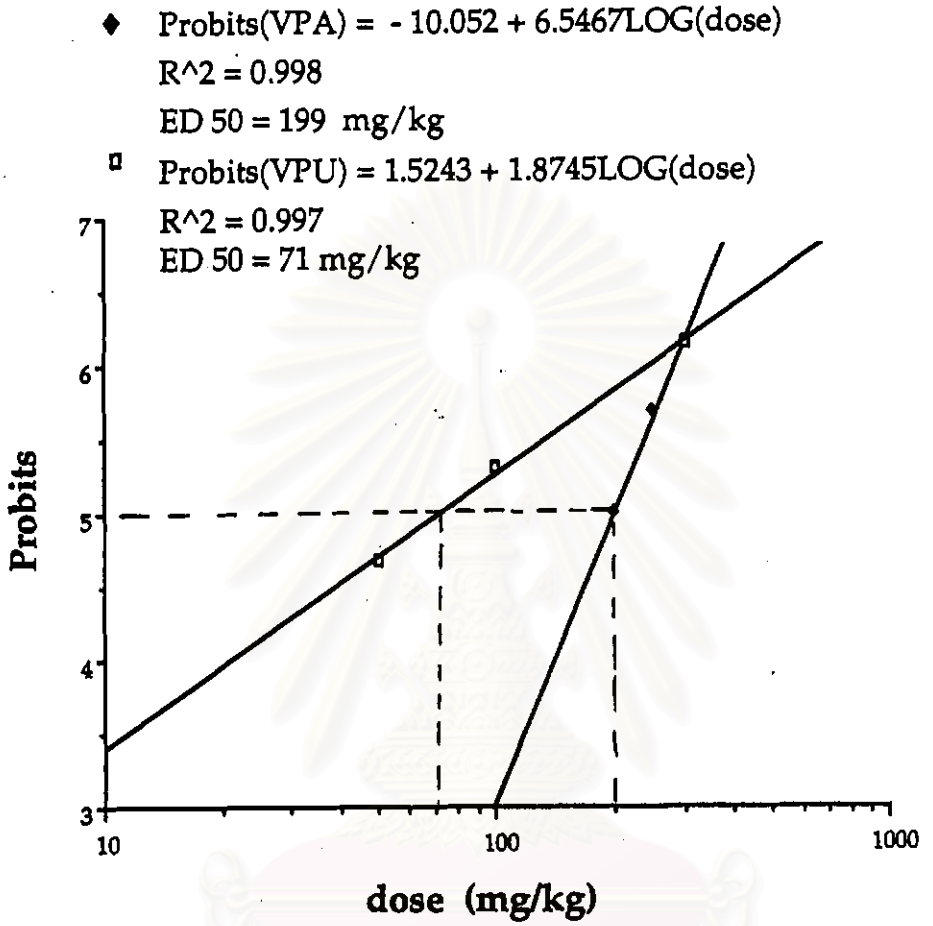


Figure 9. Log dose response curves of VPU and VPA (i.p.) on MES at 30 min pretreated time.

Table 2. ED_{50} and relative safety margin (LD_{50}/ED_{50}) of an intraperitoneal administration of VPU and VPA at different time after dosing.

time (hr)	ED_{50} (mg/kg B.W.)		Relative safety magin (LD^{*}_{50}/ED_{50})	
	VPU	VPA	VPU	VPA
1	75	216	20.70	3.87
3	104	190	14.93	4.41
6	287	339	5.41	2.47
9	426	486	3.64	1.72
12	431	565	3.60	1.48

* LD_{50} was taken from Thongchai Sooksawate (1995). They were 1553 and 838 mg/kg B. W. for VPU and VPA respectively.

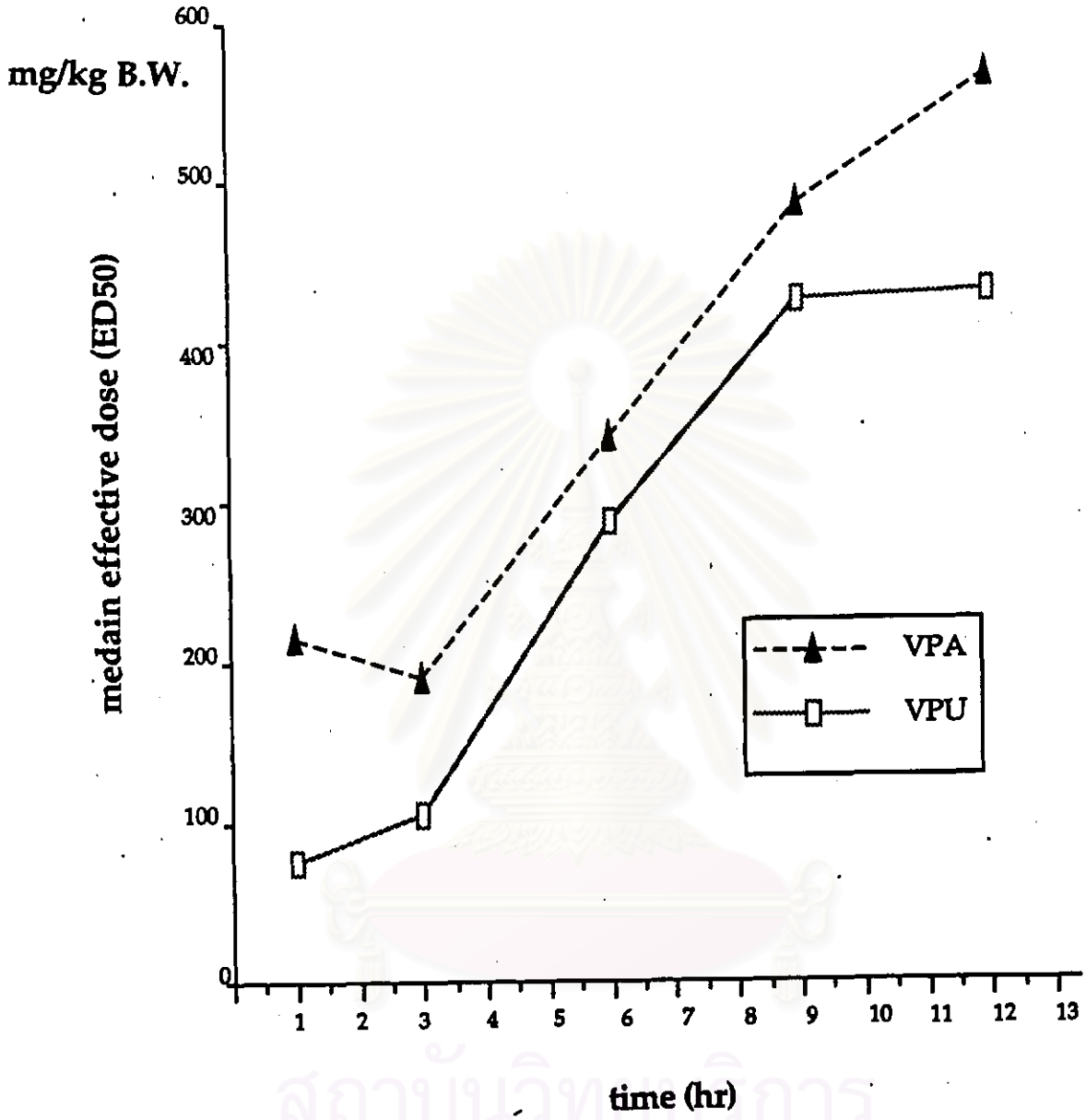
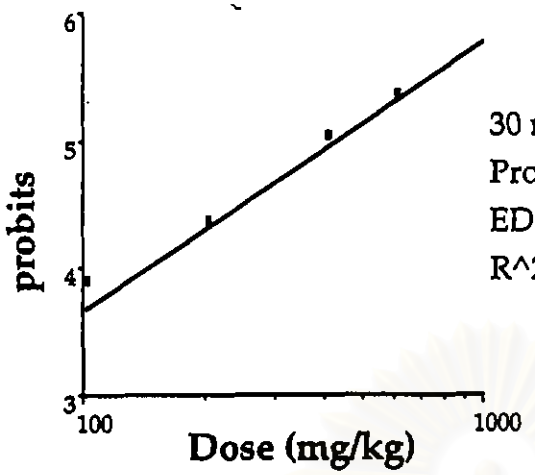
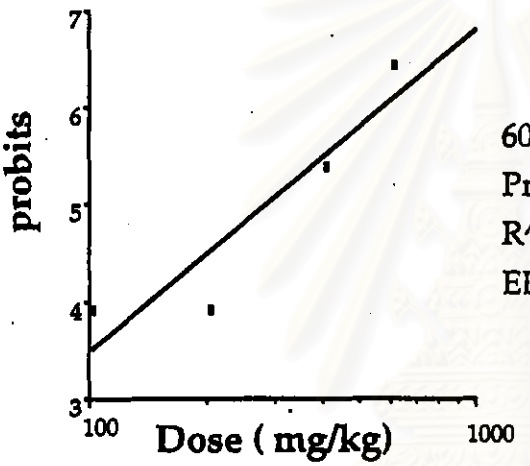


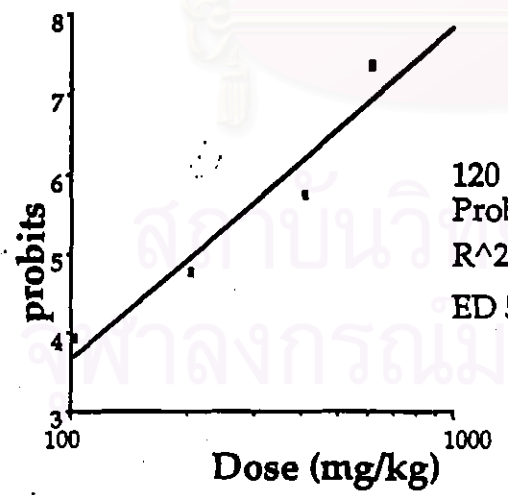
Figure 10. Protection against MES exhibited by VPU and VPA at various pretreated time in mice.



30 min.
Probits = - 3.8877e-2 + 1.9264Log(dose)
ED 50 = 413 (252-814)
R^2 = 0.995

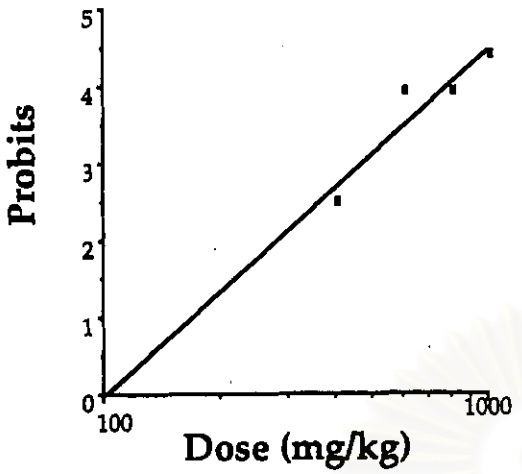


60min.
Probits = - 3.1940 + 3.3226Log(dose)
R^2 = 0.857
ED 50 = 293 (146-585)



120 min.
Probits = - 4.6533 + 4.1413Log(dose)
R^2 = 0.929
ED 50 = 215(145-315)

Figure 11. Log dose response curves of VPU (p.o.) on MES at 30, 60 and 120 min pretreated times.

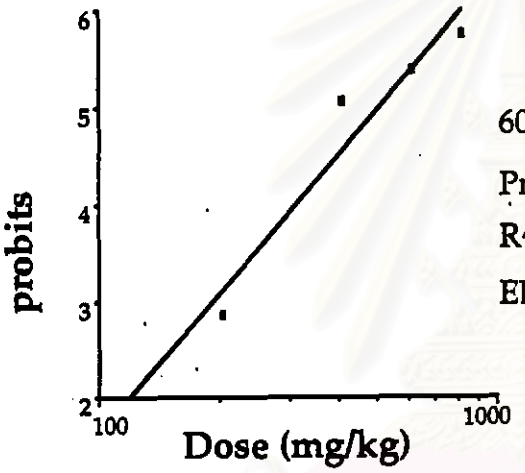


30 min.

Probits = - 9.1209 + 4.5151Log (dose)

R^2 = 0.879

ED 50 = 1341 (944-1940)

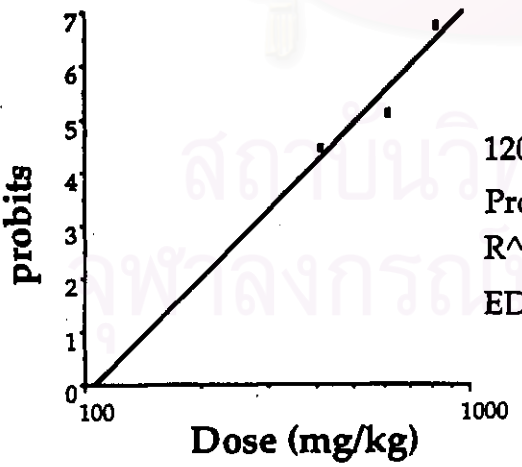


60 min.

Probits = - 7.9000 + 4.7615Log(dose)

R^2 = 0.923

ED 50 = 512 (391-671)



120 min.

Probits = - 14.784 + 7.2797Log(dose)

R^2 = 0.892

ED 50=522 (380-716)

Figure 12. Log dose response curves of VPA (p.o.) on MES at 30, 60 and 120 min pretreated times.

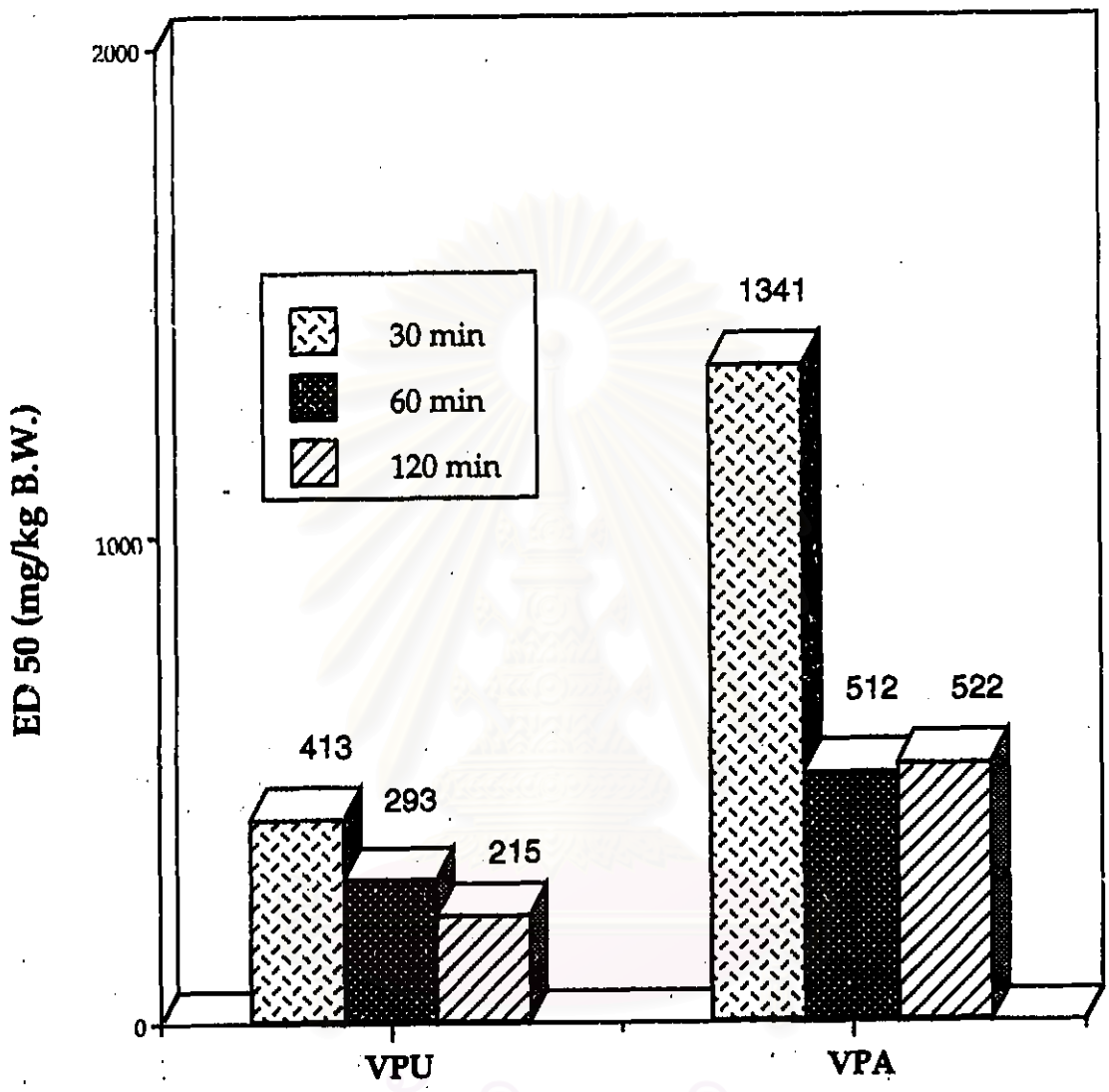


Figure 18. Comparison of ED_{50} at various pretreated times of orally given VPU and VPA against MES in mice.

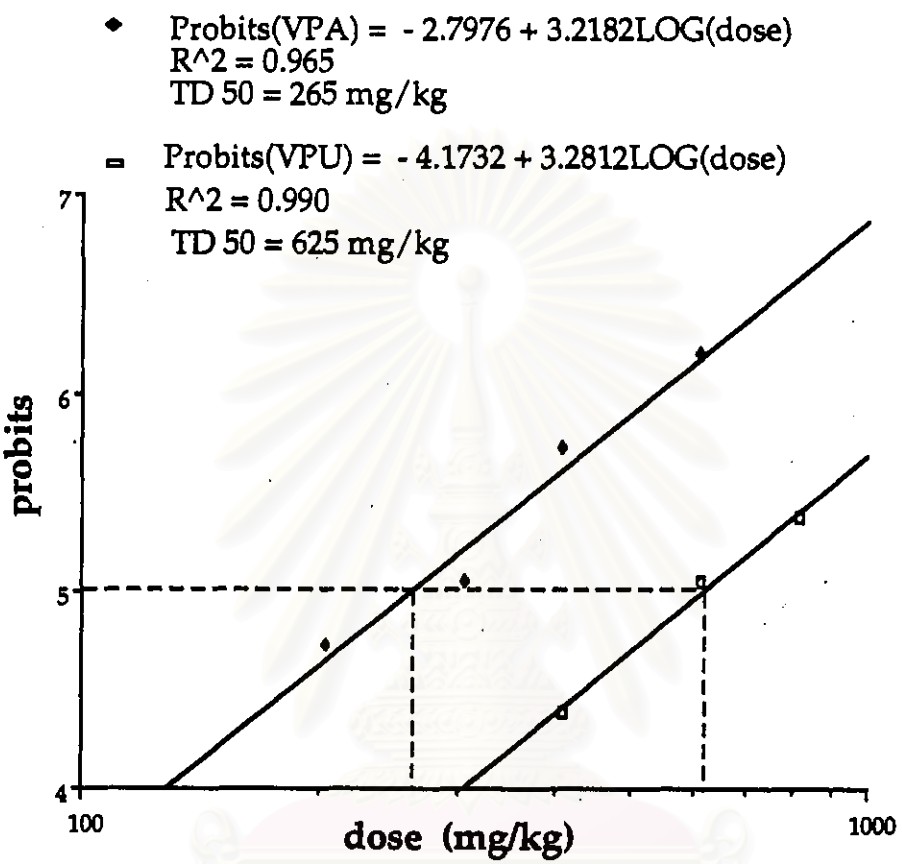


Figure 14. Log dose response curves of neurotoxicity exhibited by VPU and VPA (i.p.) in mice.

Table 3. ED_{50} ,TD_{50} and PI (TD_{50}/ED_{50}) of an intraperitoneal administration of VPU and VPA.

parameter	animal model	VPU	VPA
ED_{50} (mg/kg B.W.)	MES	71	199
TD_{50} (mg/kg B.W.)	Rotorod	625	265
PI	-	8.8	1.3

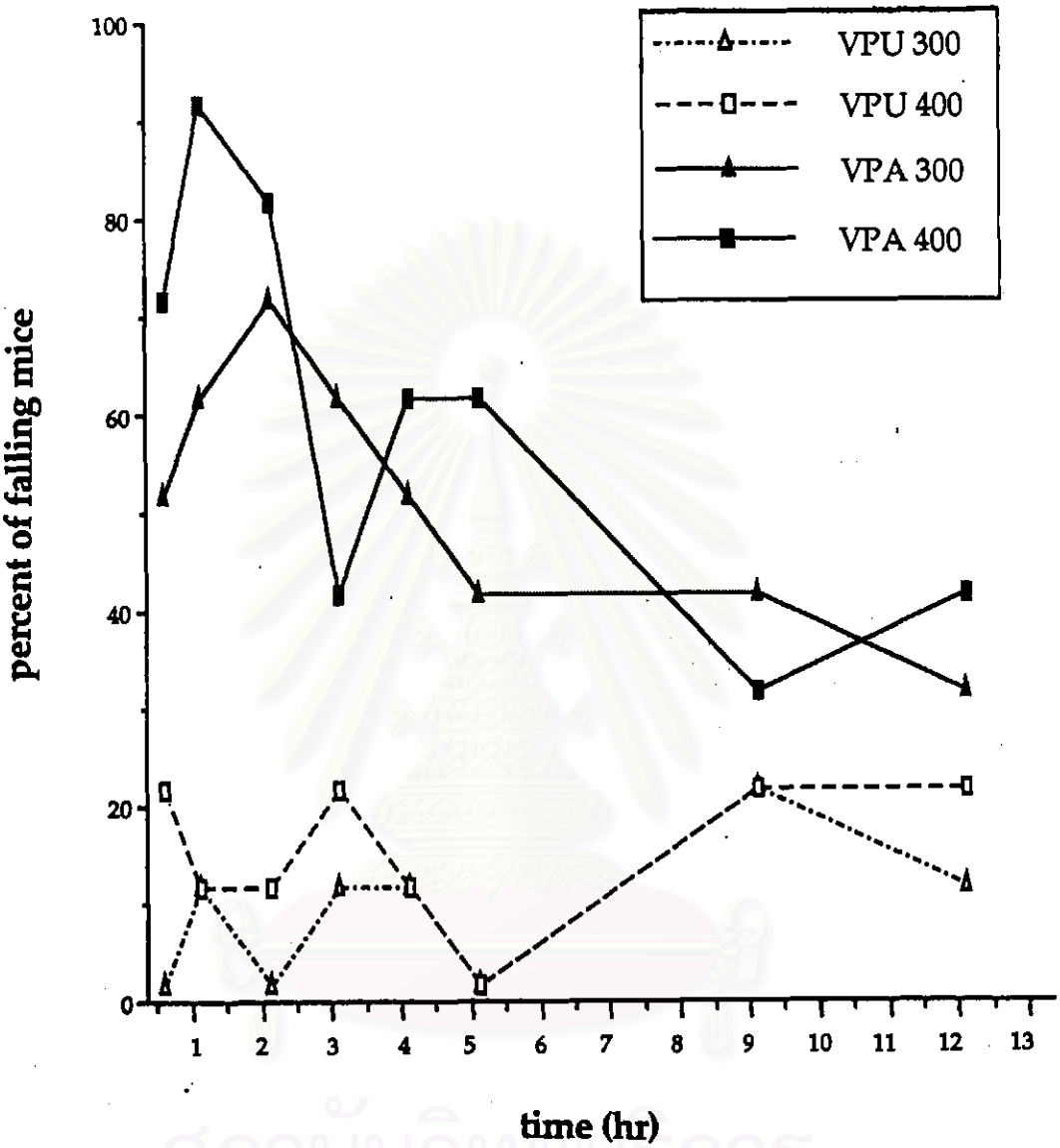


Figure 15. Neurotoxicity of VPU and VPA by rotorod test at various pretreated times in mice.

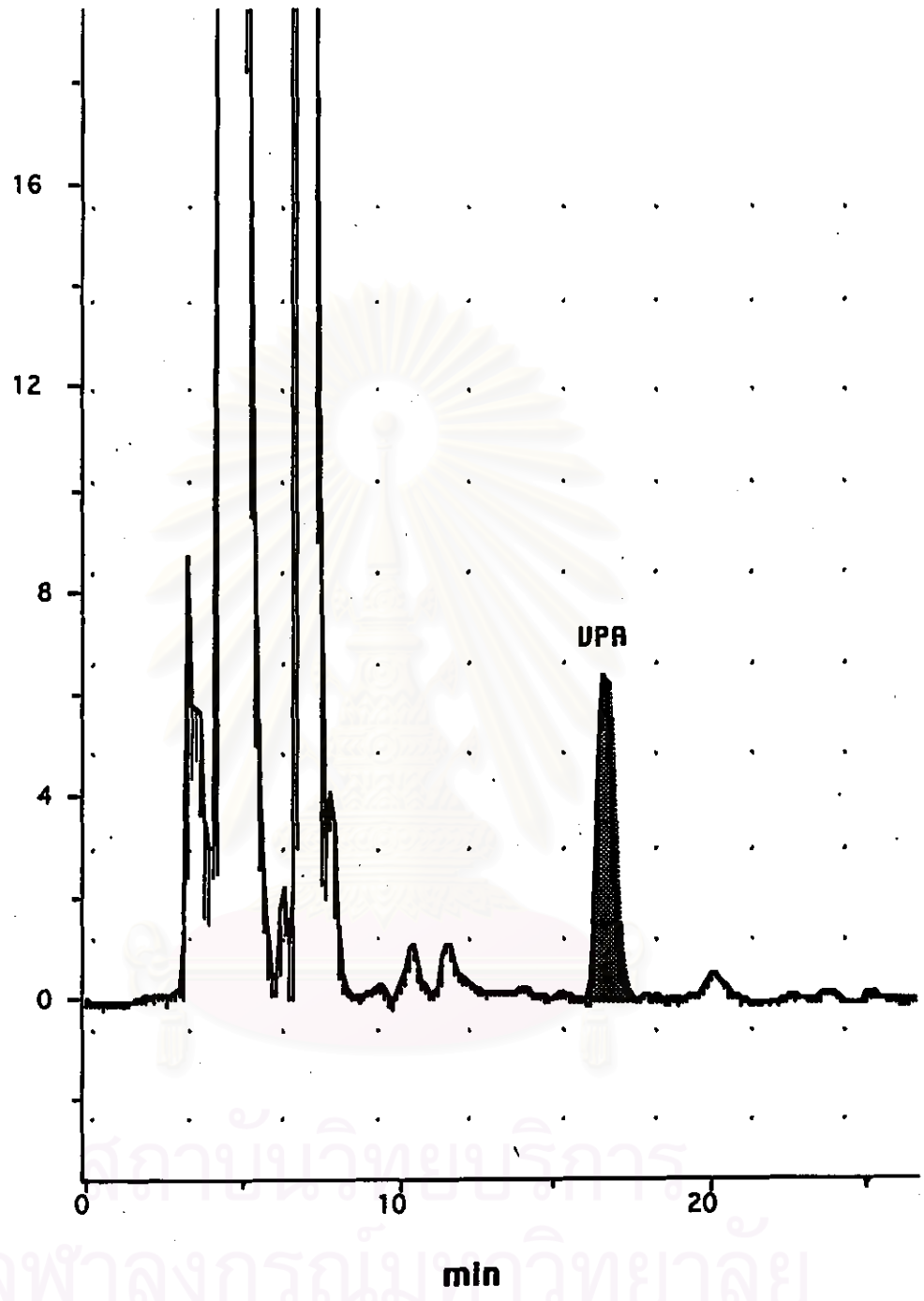


Figure 16. HPLC chromatogram of ADAM-derivatized standard VPA.

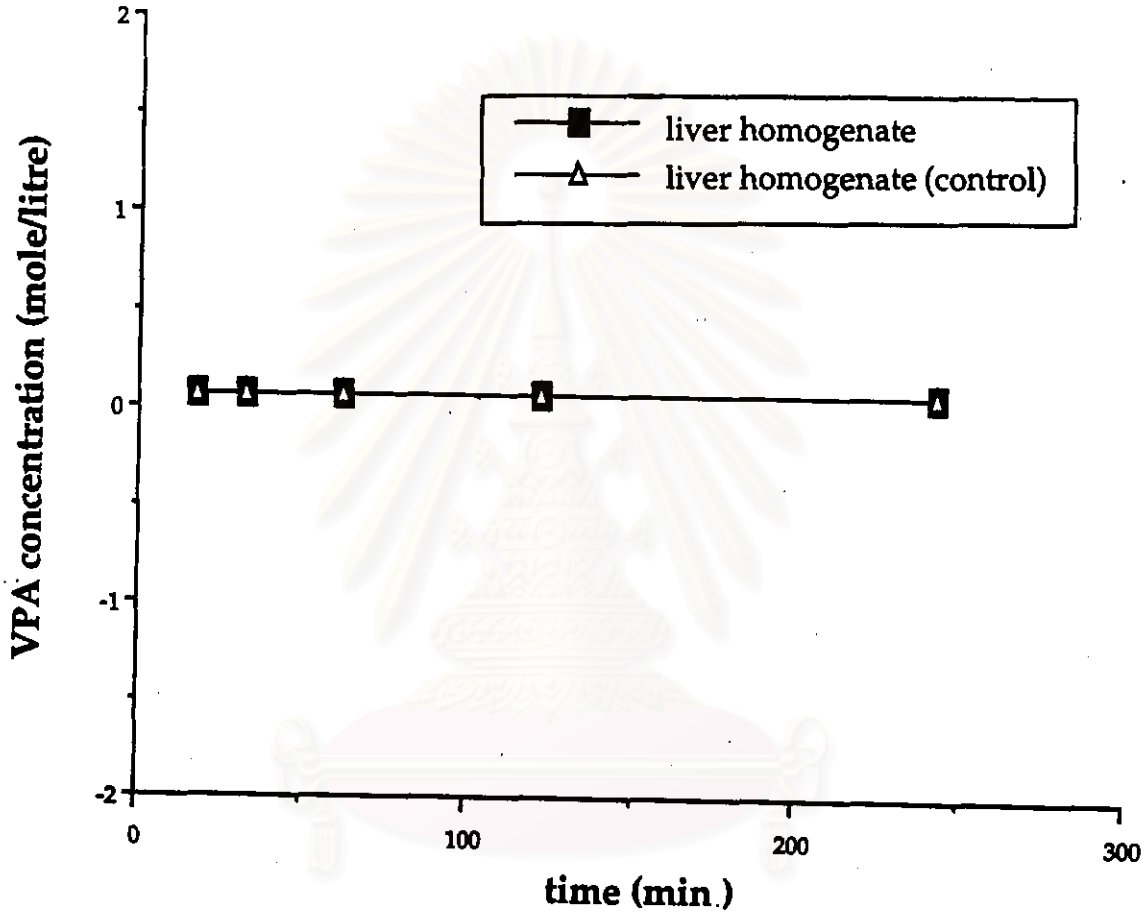


Figure 17. VPA concentration in rat's liver homogenate at various incubation times after the administration of VPU at time 0.

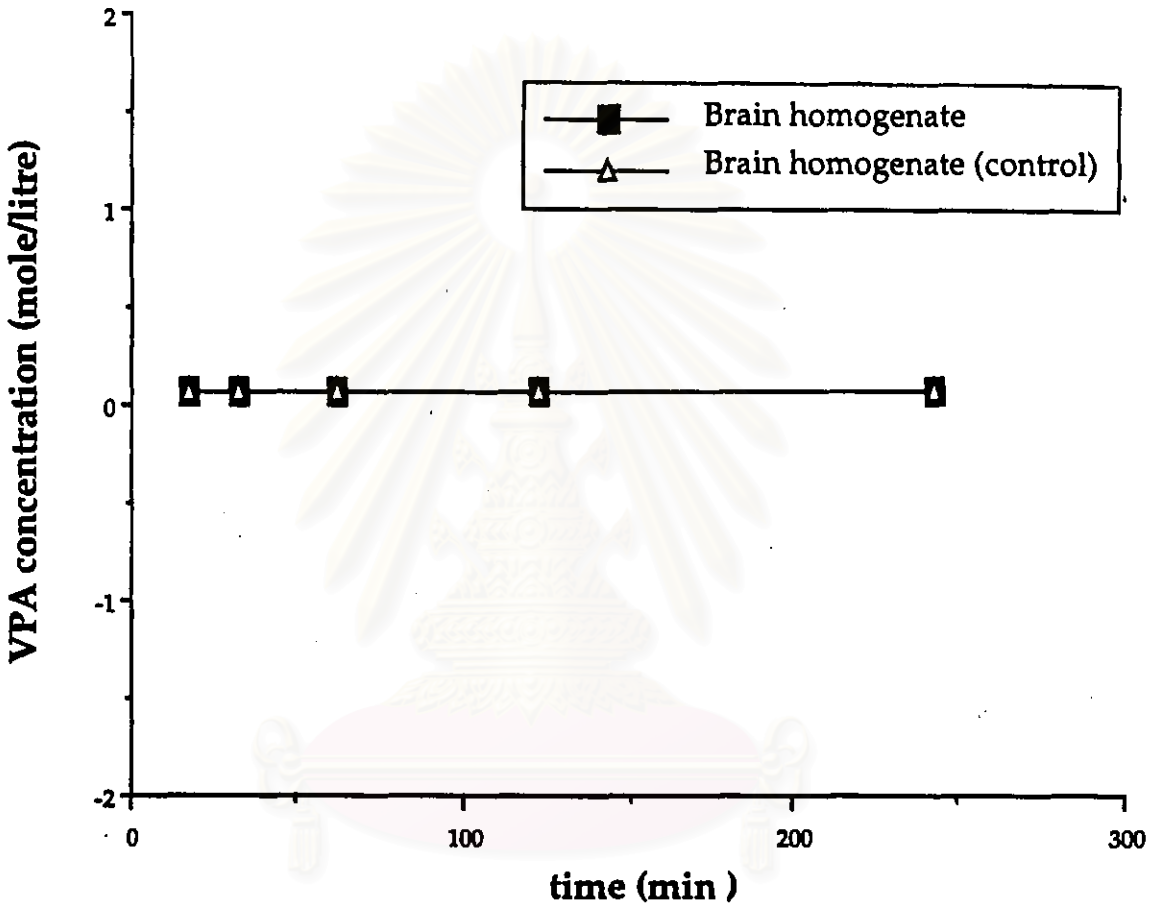


Figure 18. VPA concentration in rat's brain homogenate at various incubation times after the administration of VPU at time 0.

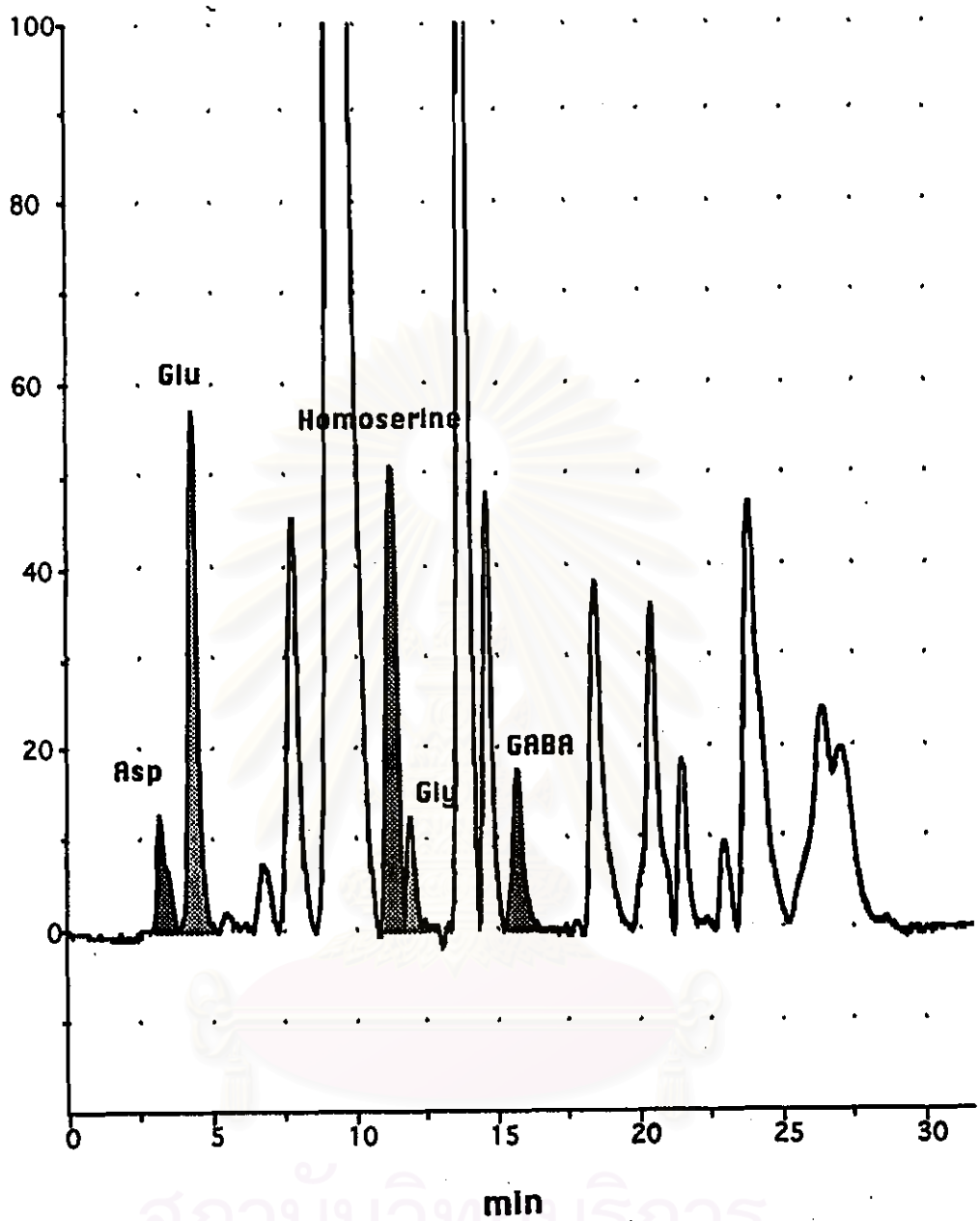


Figure 19. HPLC chromatogram of OPA-derivatized amino acids from the rat cerebral cortex.

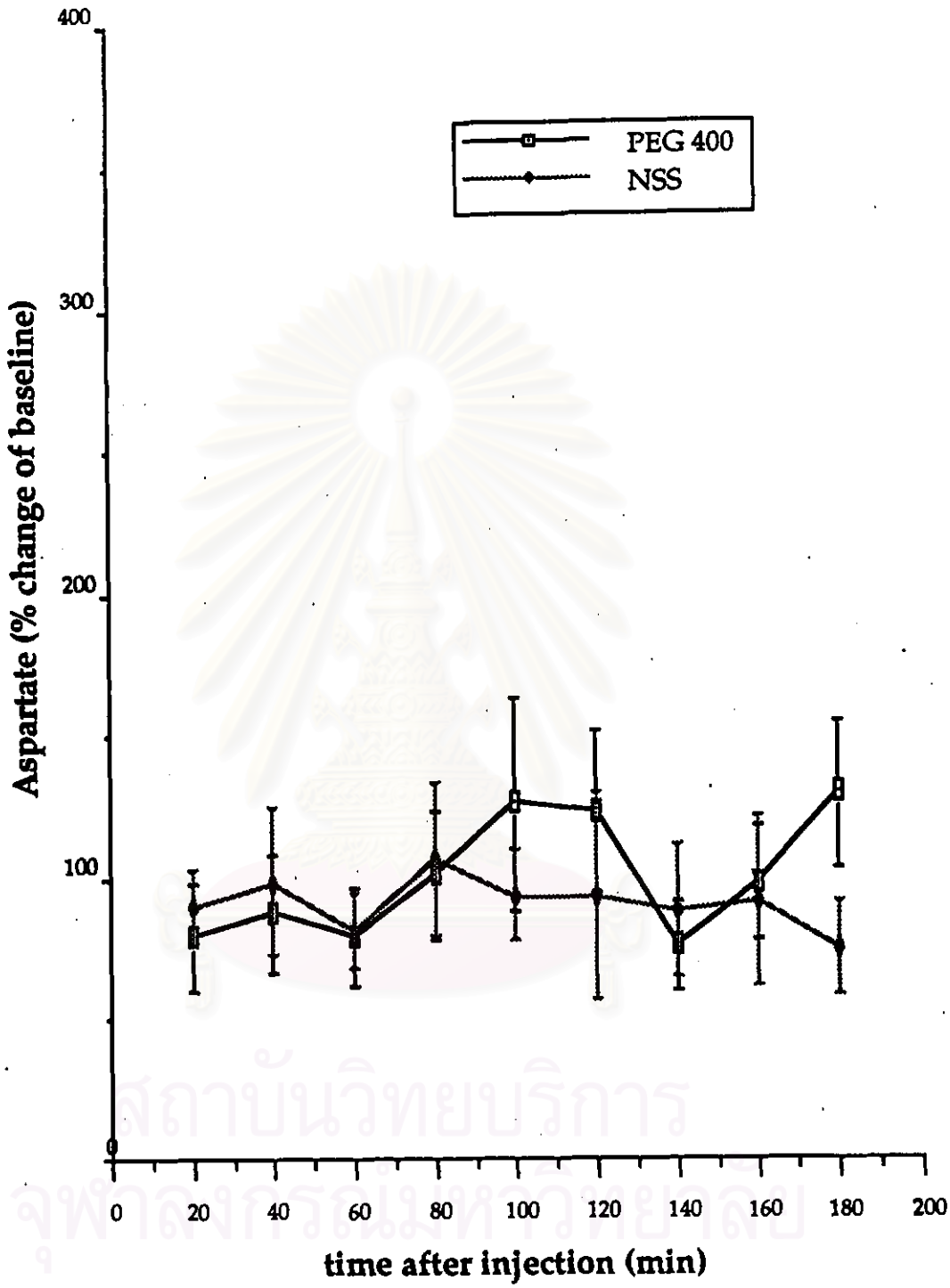


Figure 20. Change in the rat cortical aspartate levels at various times after an intraperitoneal administration of NSS and PEG400.

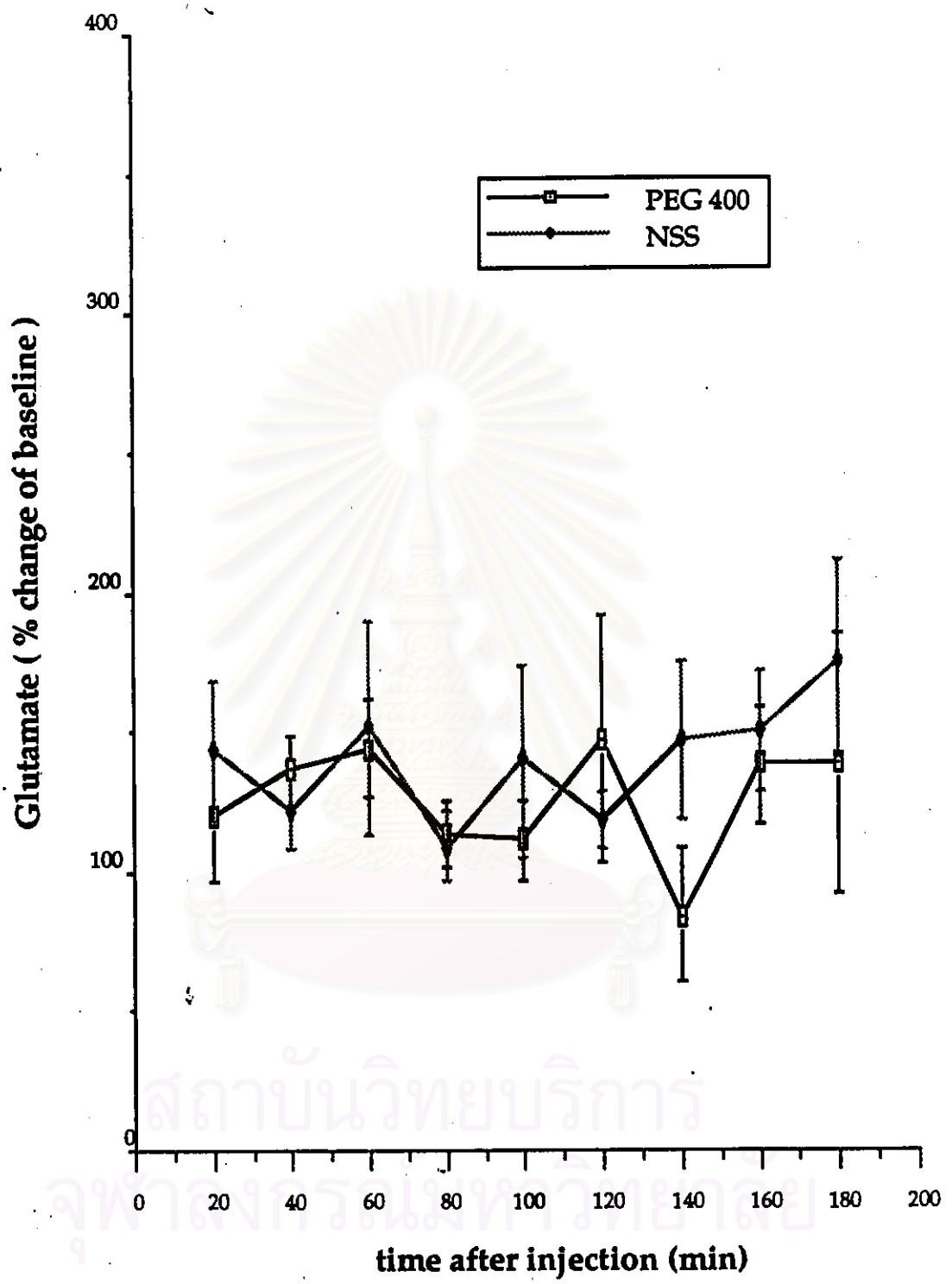


Figure 21. Change in the rat cortical glutamate levels at various times after an intraperitoneal administration of NSS and PEG400.

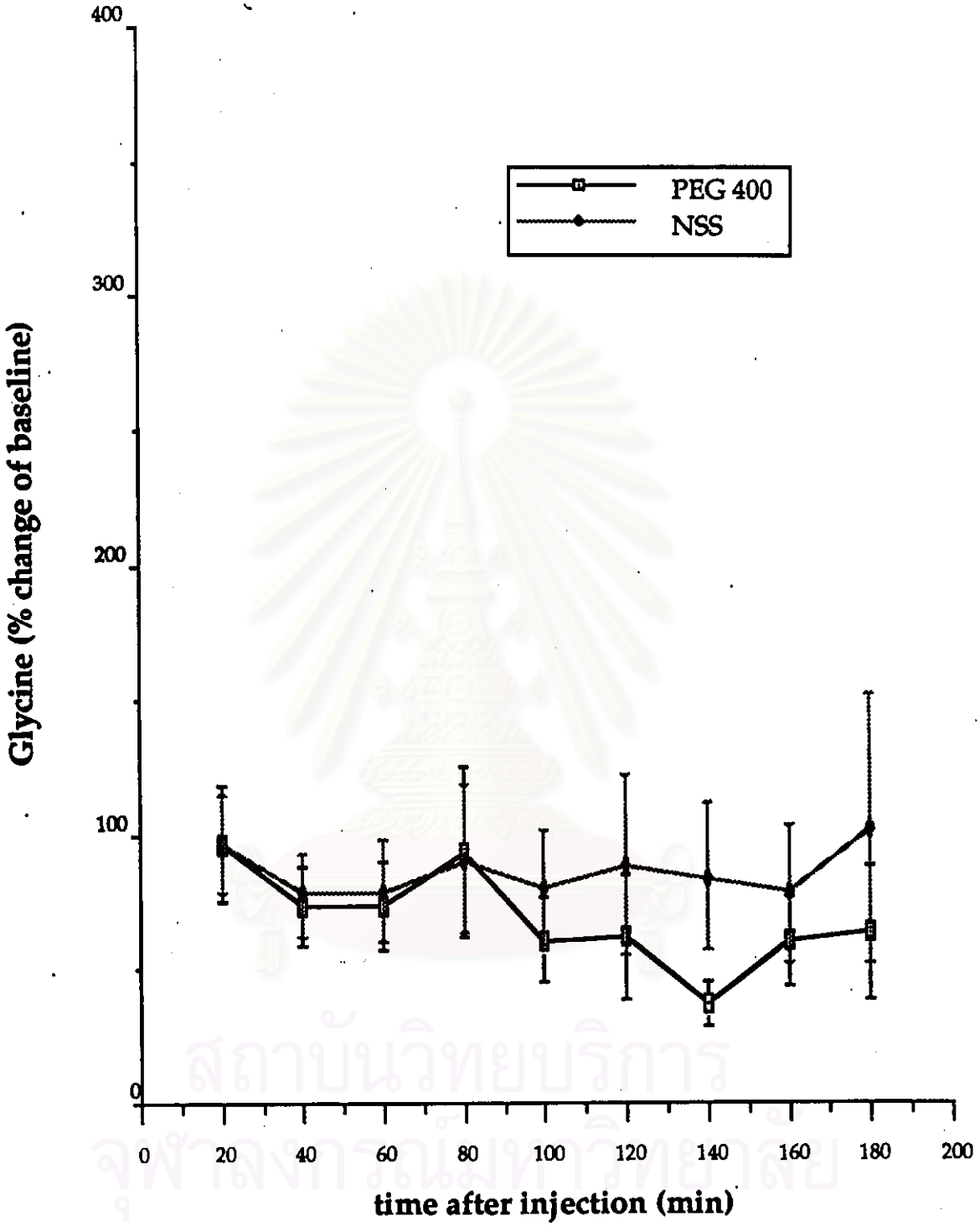


Figure 22. Change in the rat cortical glycine levels at various times after an intraperitoneal administration of NSS and PEG400.

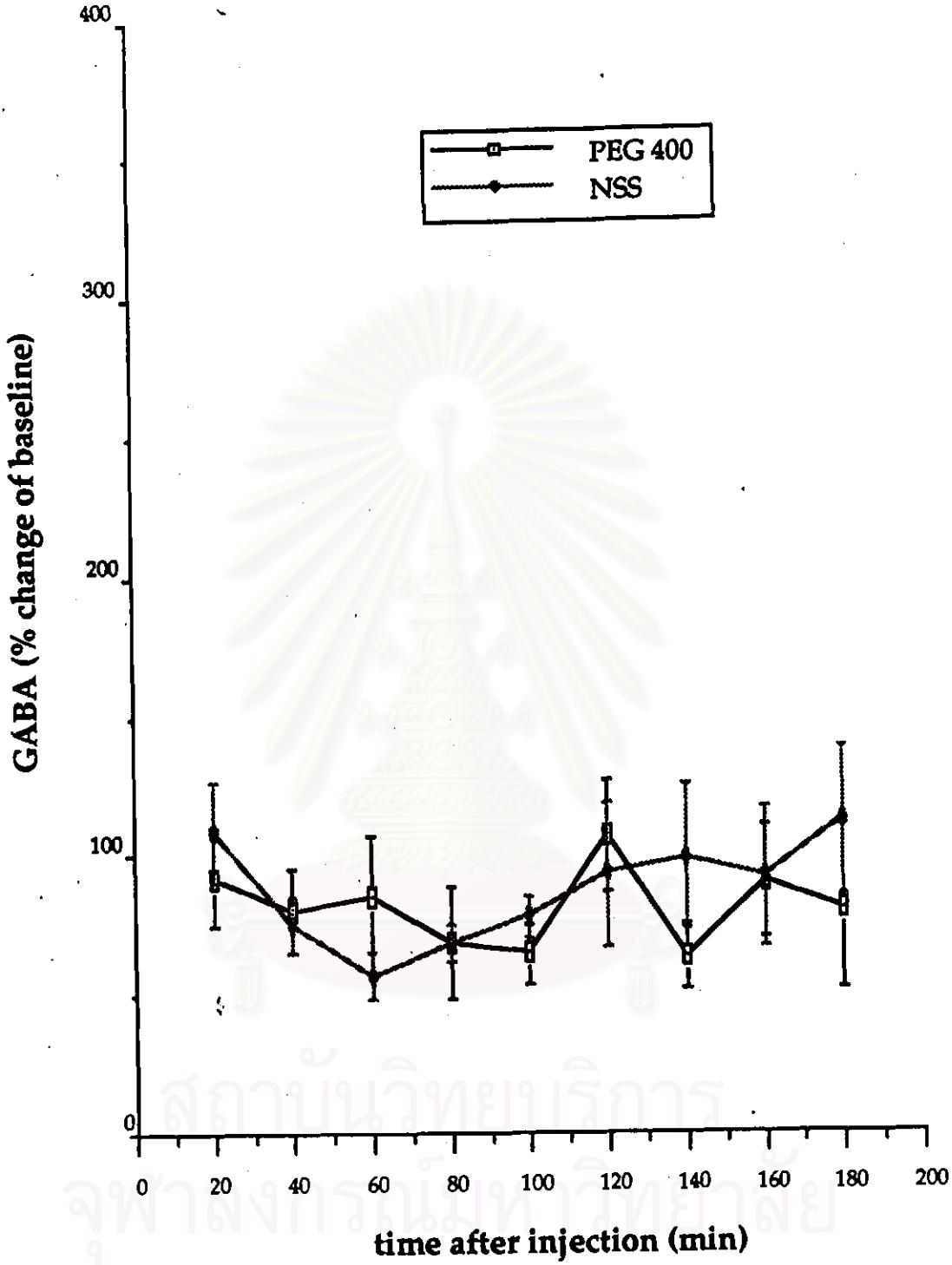


Figure 23. Change in the rat cortical GABA levels at various times after an intraperitoneal administration of NSS and PEG400.

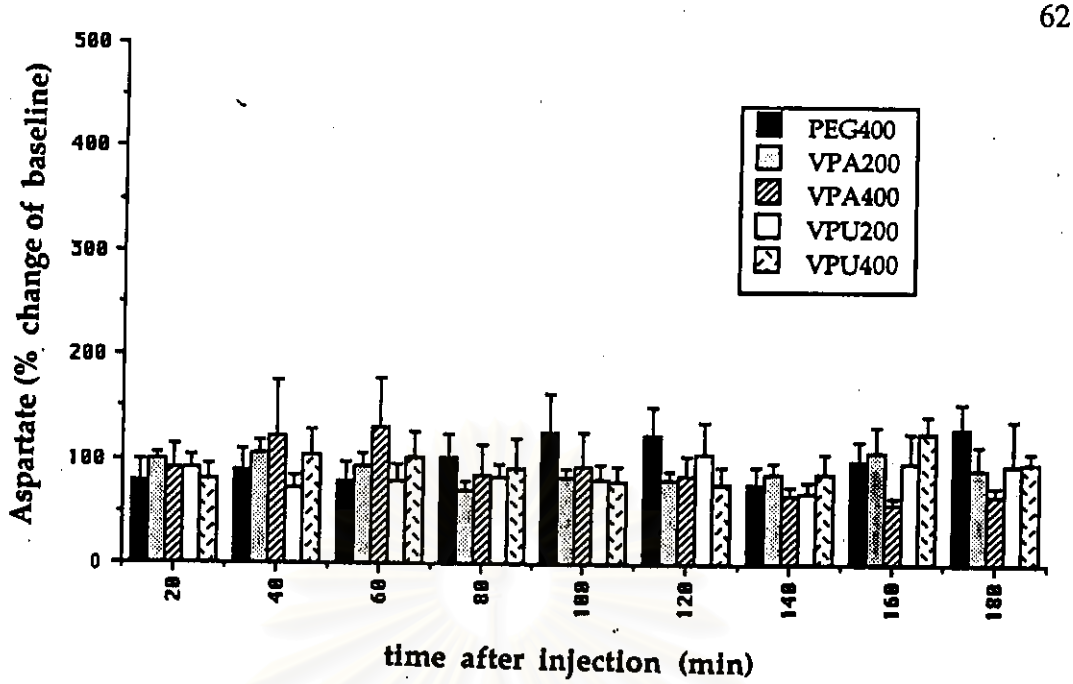


Figure 24. Effect of an intraperitoneal administration of VPA and VPU on the rat cortical aspartate levels at various times.

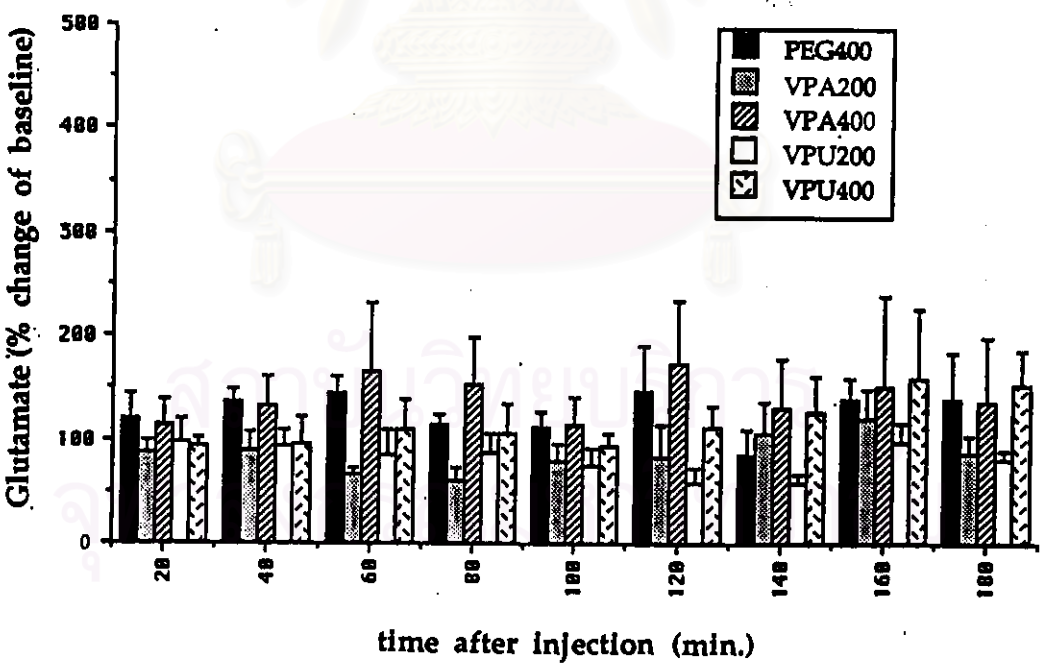


Figure 25. Effect of an intraperitoneal administration of VPA and VPU on the rat cortical glutamate levels at various times.

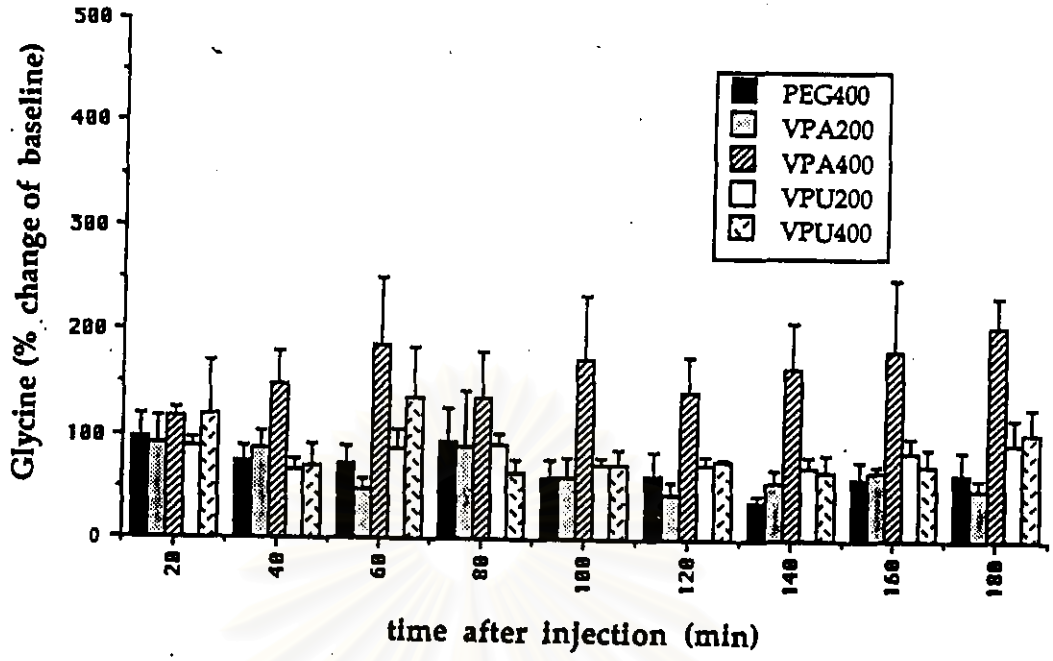


Figure 26. Effect of an intraperitoneal administration of VPA and VPU on the rat cortical glycine levels at various times.

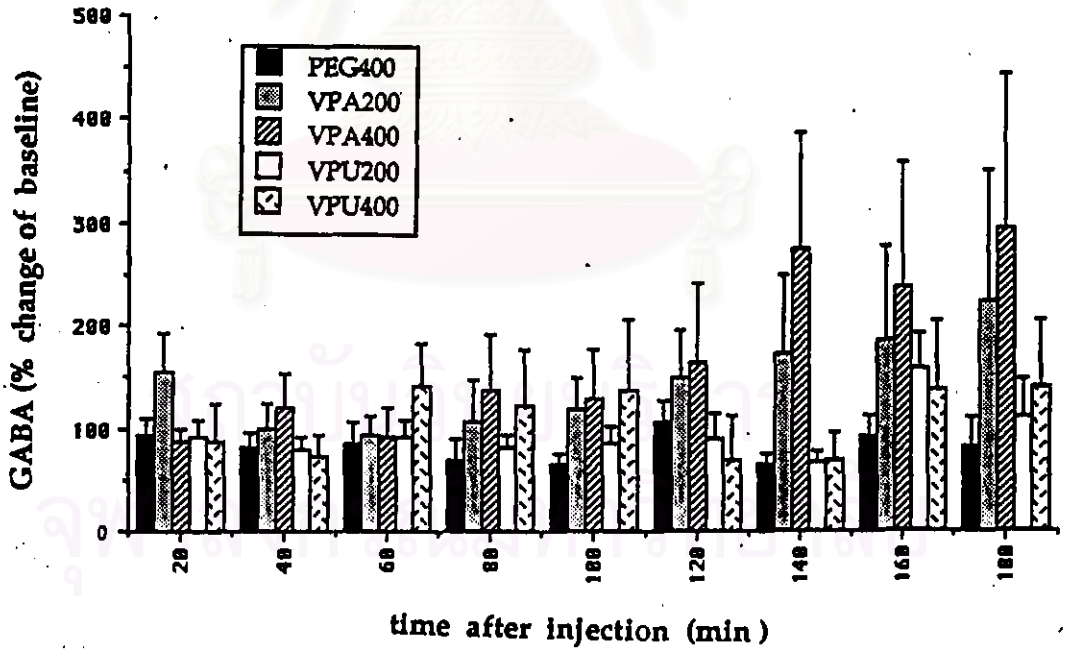


Figure 27. Effect of an intraperitoneal administration of VPA and VPU on the rat cortical GABA levels at various times.

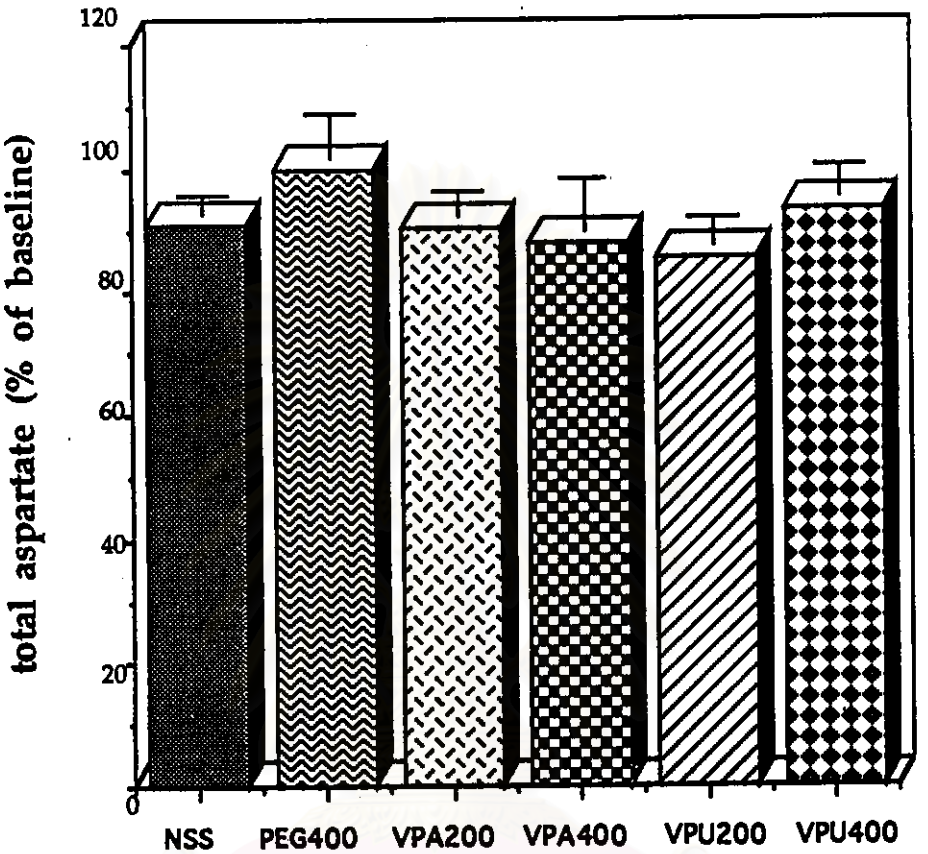
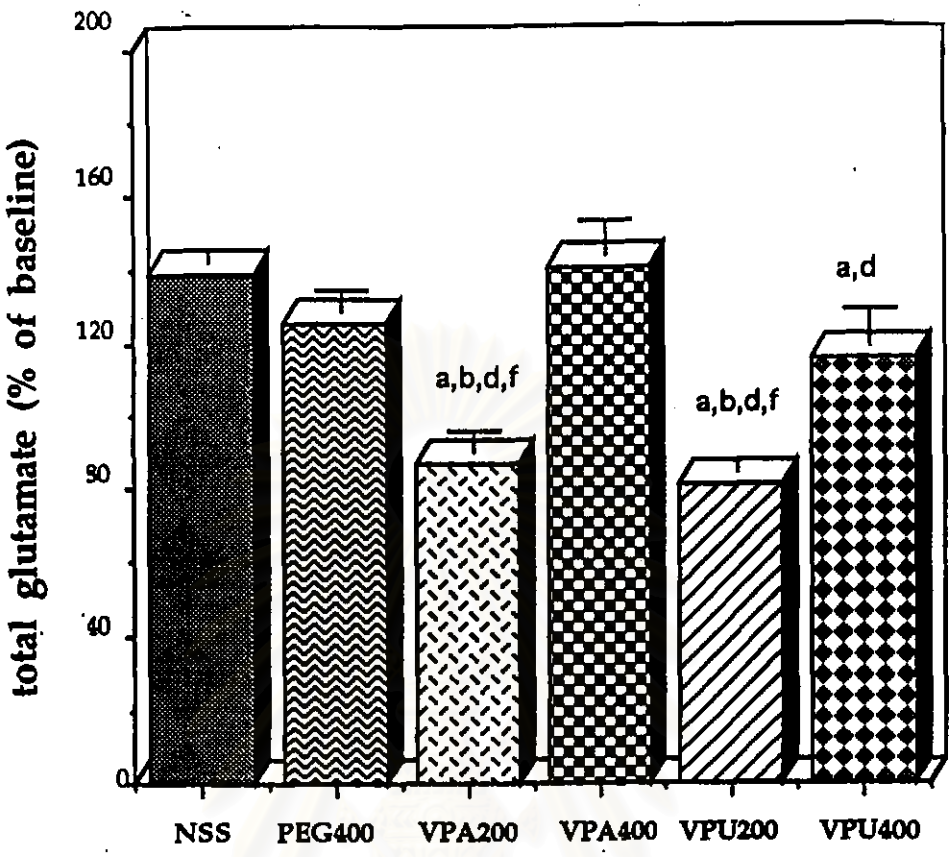


Figure 28. Effect of VPA and VPU on the total amount of the rat cortical aspartate in the dialysate collected for 3 hours after an intraperitoneal administration of the test substances.



[a] p<0.05 denotes statistically significant from NSS

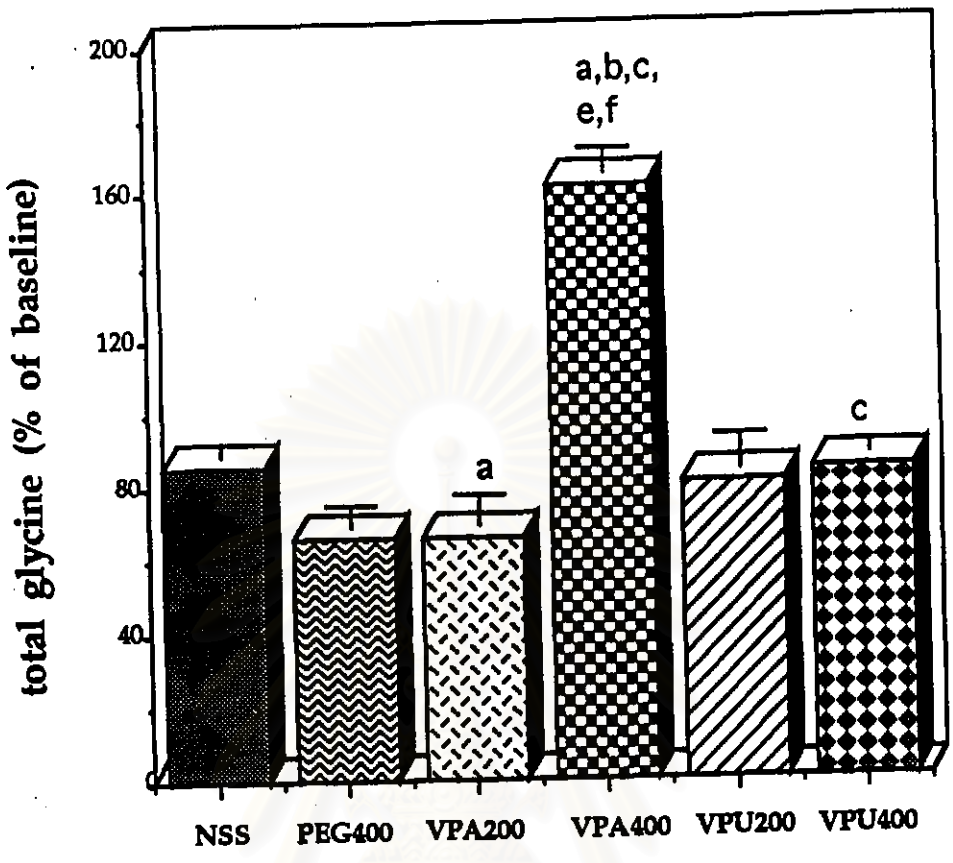
[b] p<0.05 denotes statistically significant from PEG400

[d] p<0.05 denotes statistically significant from VPA400

[f] p<0.05 denotes statistically significant from VPU400

n = 4-6

Figure 29. Effect of VPA and VPU on the total amount of the rat cortical glutamate in the dialysate collected for 3 hours after an intraperitoneal administration of the test substances.



[a] p<0.05 denotes statistically significant from NSS

[b] p<0.05 denotes statistically significant from PEG400

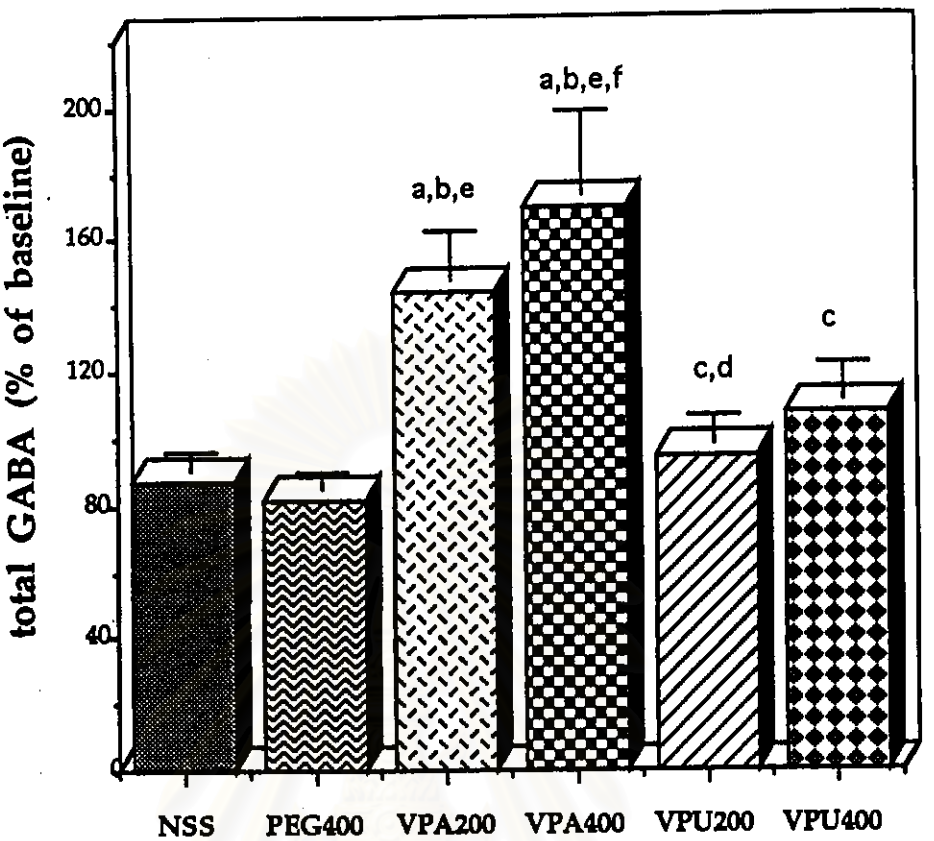
[c] p<0.05 denotes statistically significant from VPA200

[e] p<0.05 denotes statistically significant from VPU200

[f] p<0.05 denotes statistically significant from VPU400

n = 4-6

Figure 30. Effect of VPA and VPU on the total amount of the rat cortical glycine in the dialysate collected for 3 hours after an intraperitoneal administration of the test substances.



[a] p<0.05 denotes statistically significant from NSS

[b] p<0.05 denotes statistically significant from PEG400

[c] p<0.05 denotes statistically significant from VPA200

[d] p<0.05 denotes statistically significant from VPA400

[e] p<0.05 denotes statistically significant from VPU200

[f] p<0.05 denotes statistically significant from VPU400

n = 4-6

Figure 31. Effect of VPA and VPU on the total amount of the rat cortical GABA in the dialysate collected for 3 hours after an intraperitoneal administration of the test substances.