Chapter III

Results

1. Anticonvulsant activity.

Anticonvulsant activity of Amide 1C was observed when it was given either intraperitoneally or orally. Intraperitoneally given Amide 1 C has demonstrated a dose related anticonvulsant in all animal models tested except in the convulsion induced by strychnine. Similar results with lower degree of protection were observed in VPA treated animals. While PEG 400 (0.1 ml/ 25 g BW.), which was given to control group exhibited no protection. In comparison, Amide 1C produced higher potency than VPA about 1, 3 and 1 times in MES, PTZ and bicuculline tests respectively. Furthermore, Amide 1C was demonstrating peak effect at 15 min while it was 30 min for VPA.

1.1 Anticonvulsant activity against MES test.

1.1.1 Intraperitoneal route

As illustrated in Figure 7 and 8.,an intraperitoneal administration of Amide 1C and VPA demonstrated protection against MES in mice in a dose dependent manner.

The ED_{50} of Amide 1C were 81, 92 and 257 mg/kg BW. at pretreated time of 15, 30 and 60 min, respectively, while corresponding values for VPA were 266, 218 and 212 mg/kg BW. (Figure 9.).

The optimal pretreated time defined as the minimal time for the test substance to exert its highest anticonvulsant activity was found to be 15 min for Amide 1C and 30 min for VPA. As shown in Figure 9., the ED_{50} of Amide 1C and VPA at optimal pretreated time were 81 and 218 mg/kg BW. respectively and they would be subsequently used in other experiments.

1.1.2 Oral route.

Because of the limited supply of Amide 1C, the ED_{50} of an orally administration in MES test cannot be determined. However, its orally effective has been demonstrated at dose of 400 mg/kg BW. (100% protection, n = 4) in 15 min after administration while the ED_{50} of VPA was 250-330 mg/kg BW. (Shuto and Nishigaki, 1970; Loscher et al, 1984; Ferrendelli et al, 1989) (Table 3.).

1.2 Anticonvulsant activity against PTZ seizure.

In PTZ test, intraperitoneal injection of Amide 1C and VPA in mice exhibited anticonvulsant against PTZ seizure in a dose dependent manner similar to MES test.

As shown in the Figure 10., the ED_{50} of amide 1C and VPA at optimal pretreated time were 33 and 93 mg/kg BW. respectively.

1.3 Anticonvulsant activity against bicuculline and strychnine convulsion in mice.

As shown in Figure 11., both Amide 1C and VPA exerted anticonvulsant activity against bicuculline induced convulsion. The ED_{50} of Amide 1C and VPA were 214 and 395 mg/kg BW. respectively whereas neither Amide 1C (300 mg/kg BW.) nor VPA (600 mg/kg BW.) were found to be effective in strychnine-induced convulsion (Table 3.).

2. Toxicity

2.1 Acute toxicity

The most frequent clinical signs observed in mice receiving high dose of tested substances were ataxia, sedation, hypnosis, and respiratory tract secretion.

The data of mortality, was evaluated by determination of the median lethal dose (LD_{50}) following a single intraperitoneal administration of Amide 1C and VPA in 72 hrs. However, most of death occured in 24 hrs after dosing. As shown in Figure 12., the LD_{50} of Amide 1C and VPA were 602 and 832 mg/kg BW. respectively.

The relative safety margin (LD_{50}/ED_{50}) of Amide 1C in MES and PTZ seizure test were 7.43 and 18.25, while the corresponding value for VPA were 3.82 and 8.95 respectively (Table 4.).

2.2 Rotorod test

The neurological impairment expressed as percent of falling mice on rotorod test after intrapertoneal administration of various doses of Amide 1C and VPA has been shown in Figure 13. Neurological deficit observed only in Amide 1C and VPA but not PEG 400 treated group. As illustrated in Figure 14., both Amide 1C and VPA inhibited the rotorod performance in a dose-dependent manner, whereas PEG 400 which was used as a solvent for the test substances was devoid of this effect.

The protective index (PI) was obtained by dividing the TD_{50} by the ED_{50} . They were 3.86 and 1.77 in MES and 9.48 and 4.15 in PTZ test for Amide 1C and VPA respectively (Table 4.).

2.3 Locomotor activity

In comparison to NSS, an intraperitoneal administration of PEG 400 (0.1 ml /25 g BW.) significantly depress the locomotor activity of mice. Though, both Amide 1C and VPA tended to depress locomotor activity, no statistically significant difference was noted among the effect of PEG 400, Amide 1C (50 and 100 mg/kg BW. i.p.) and VPA (100 and 200 mg/kg BW. i.p.).

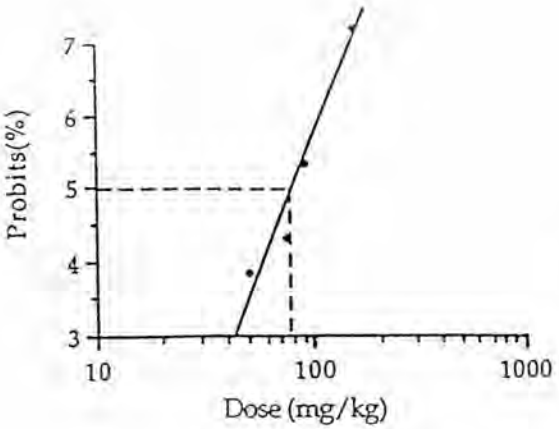
2.4 Barbiturate potentiation test

As shown in Figure 16., in comparison to PEG 400, the higher dose of both Amide 1C (100 mg/kg BW.) and VPA (200 ml/kg BW.) significantly prolonged pentobarbital sleeping time, whereas the effect of low dose of Amide 1C (50 mg/kg BW.) and VPA (100mg/kg BW.) did not differ from PEG 400.

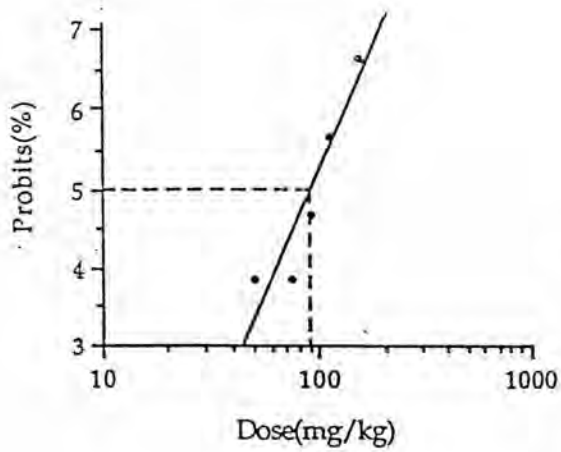
3. Effect on some cortical amino acid neurotransmitters relationto convulsion in anesthetized rats by microdialysis technique.

The effect of Amide 1C (100 and 200 mg/kg BW. i.p.) and VPA (200 and 400 mg/kg BW. i.p.) on cortical levels of aspartate, glutamate, glycine and GABA were investigated in anesthetized rats. Alteration in amino acid neurotransmitters levels was expressed as a percent change from three consecutive samples before the administration of the test substances.

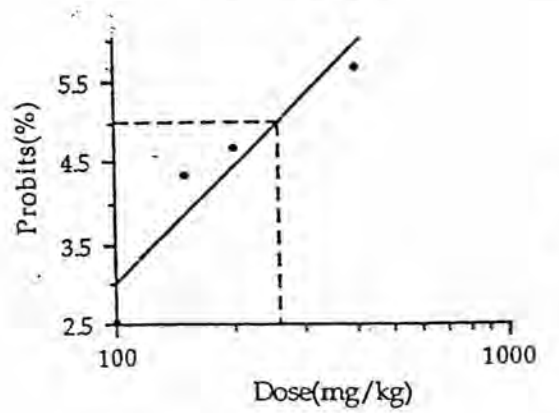
In control groups, the effect of PEG 400 on the spontaneous release of aspartate, glutamate, glycine and GABA was not statistically different from those demonstrated by NSS (figure 19 and 20.). In comparison to PEG 400, the two dose levels of Amide 1C (100 and 200 mg/kg BW.) and VPA (200 and 400 mg/kg BW.) had no significant effect on the level of all neurotransmitter measured (Figure 21, 22, 23 and 24.).



Amide 1C (15 MIN)
$y = -8.9393+7.3305*LOG(x)$, $R^2 = 0.941$
ED_{50} = 81 (65-112) mg/kg.

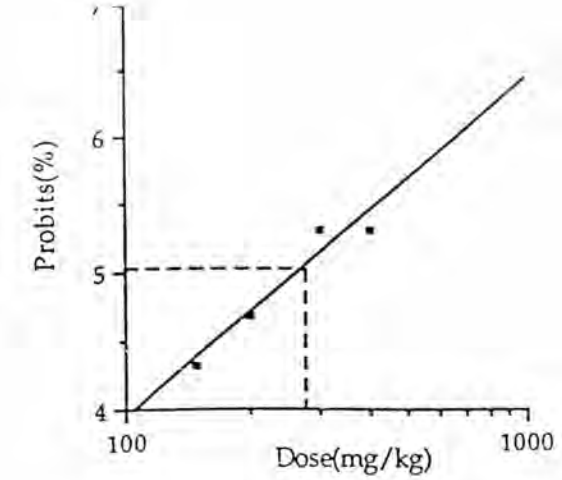


Amide 1C (30 MIN)
$y = -7.2739+6.2643*LOG(x)$, $R^2 = 0.863$
ED_{50} = 92 (70-123) mg/kg.

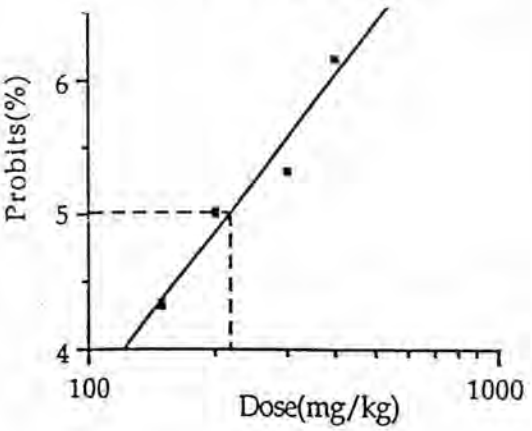


Amide 1C (60 MIN)
$y = -6.756+4.8736*LOG(x)$, $R^2 = 0.894$
ED_{50} = 257 (178-349) mg/kg.

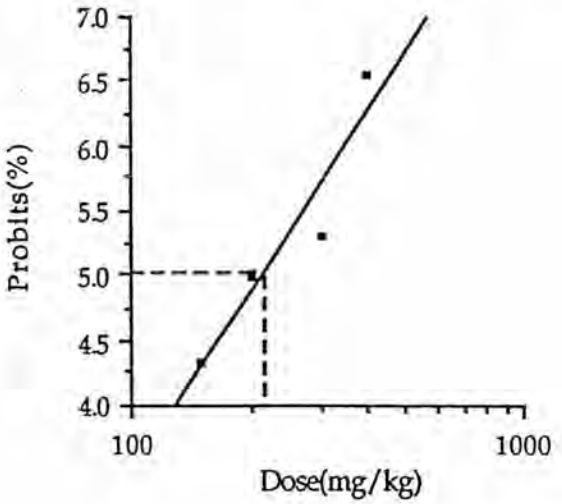
Figure 7. Log dose-response curves of Amide 1C (i.p.) in MES test at pretreated times of 15, 30 and 60 min. Numbers in parentheses represent 95% confident interval.



VPA (15 MIN)
$y = -1.1077+2.5193*LOG(x)$, $R^2 = 0.926$
ED_{50} = 266 (179-392) mg/kg.



VPA(30 MIN)
$y = -4.173+3.9227*LOG(x)$, $R^2 = 0.949$
ED_{50} = 218 (159-305) mg/kg.



VPA (60 MIN)
$y = -6.0095+4.7337*LOG(x)$, $R^2 = 0.910$
ED_{50} = 212 (160-293) mg/kg.

Figure 8. Log dose-response curves of VPA (i.p.) in MES test at pretreated times of 15, 30 and 60 min. Numbers in parentheses represent 95% confident interval.

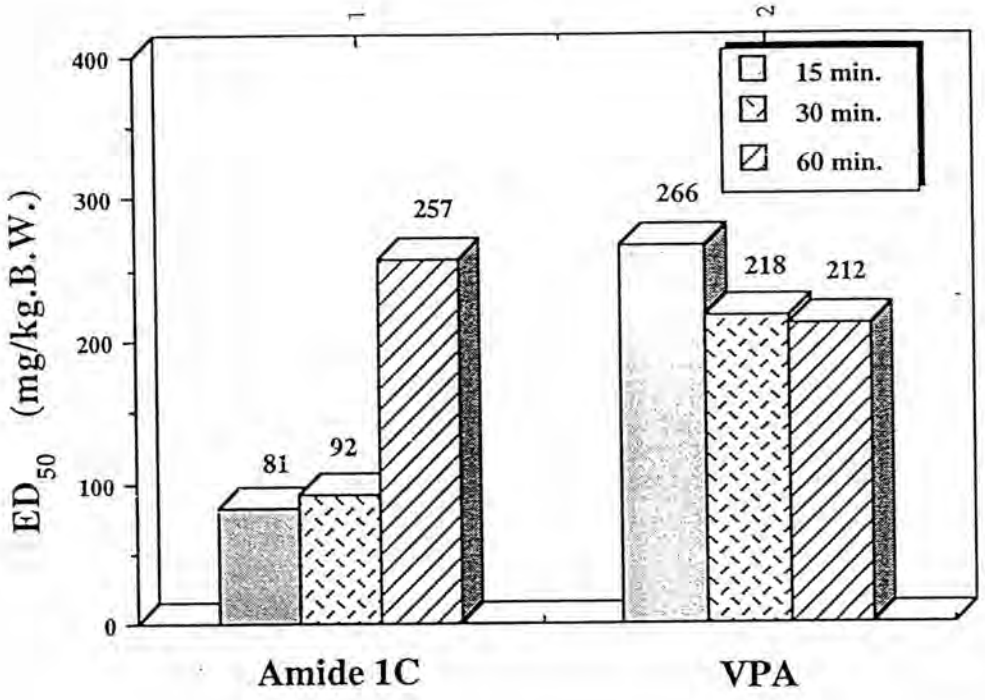
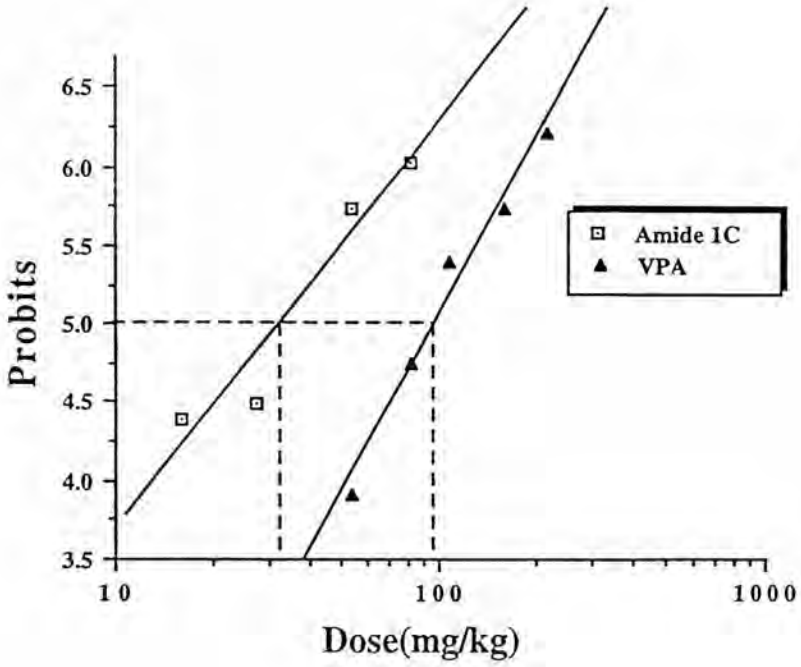


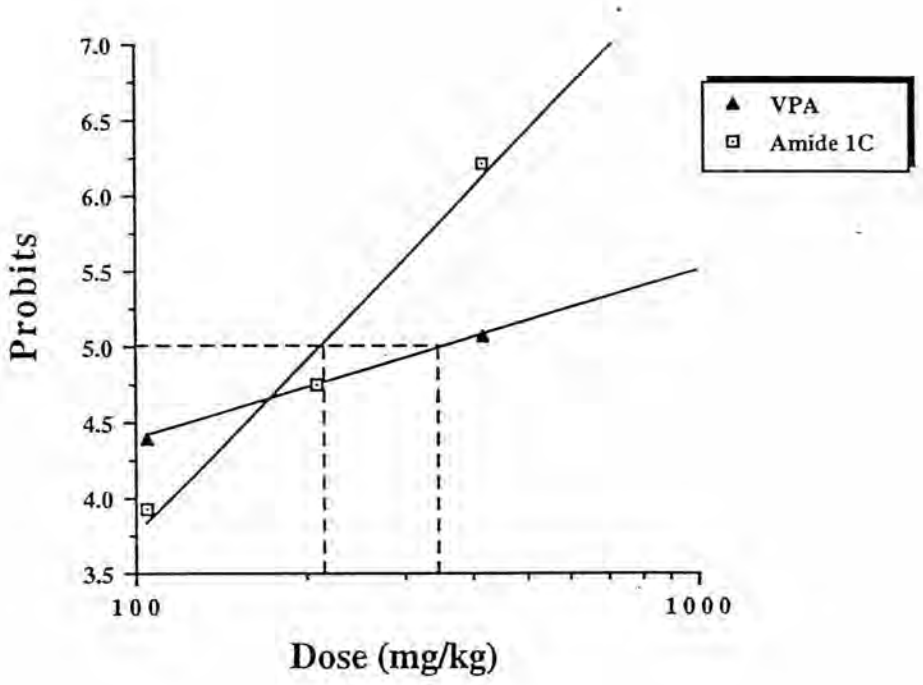
Figure 9. Comparison of ED_{50} at various pretreated times of intraperitoneally given Amide 1C and VPA in MES test in mice



Probits (Amide 1C) = 1.0961+2.6020*LOG(x), R^2 = 0 .930
ED_{50} = 33 (22-48) mg/kg.

Probits (VPA) = -2.3303+3.7136*LOG(x), R^2 = 0 .976
ED_{50} = 93 (66-132) mg/kg.

Figure 10. Log dose-response curves of Amide 1C and VPA (i.p.) in PTZ test at their resective optimal pretreated time. Numbers in parentheses represent 95% confident interval.



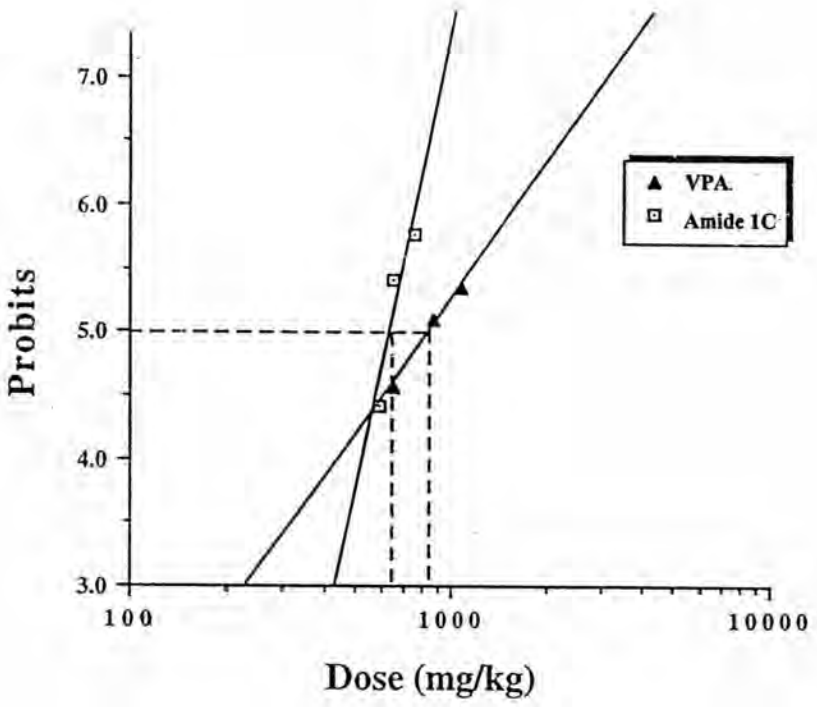
Probits (Amide 1C) = -3.8989+3.8212*LOG(x), R^2 = 0 .975
ED_{50} = 214 (152-300) mg/kg.

Probits (VPA) = 2.0911+1.1203*LOG(x), R^2 = 0 .999
ED_{50} = 395 (123-1264) mg/kg.

Figure 11. Log dose-response curves of Amide 1C and VPA (i.p.) against bicuculline induced convulsion in mice. Numbers in parentheses represent 95% confident interval.

Experimental seizures seizures	ED_{50} of test Substances (mg/kg B.W.)	
	VPA	Amide 1C
MES	218	81
PTZ	93	33
Bicuculline-induced	395	214
Strychnine-induced	> 600	> 300

Table 3. Anticonvulsant activity of intraperitoneally given Amide 1C and VPA on various animal models at optimal pretreated time of 15 and 30 min respectively.



Probits (Amide 1C) = -28.363+12.005*LOG(x), R^2 = 0 .830
LD_{50} = 602 (542-668) mg/kg.

Probits (VPA) = -5.3290+3.5382*LOG(x), R^2 = 0 .984
LD_{50} = 832 (567-1208) mg/kg.

Figure 12. Log dose-response curves of Amide 1C and VPA (i.p.) on acute toxicity (lethality) in mice.

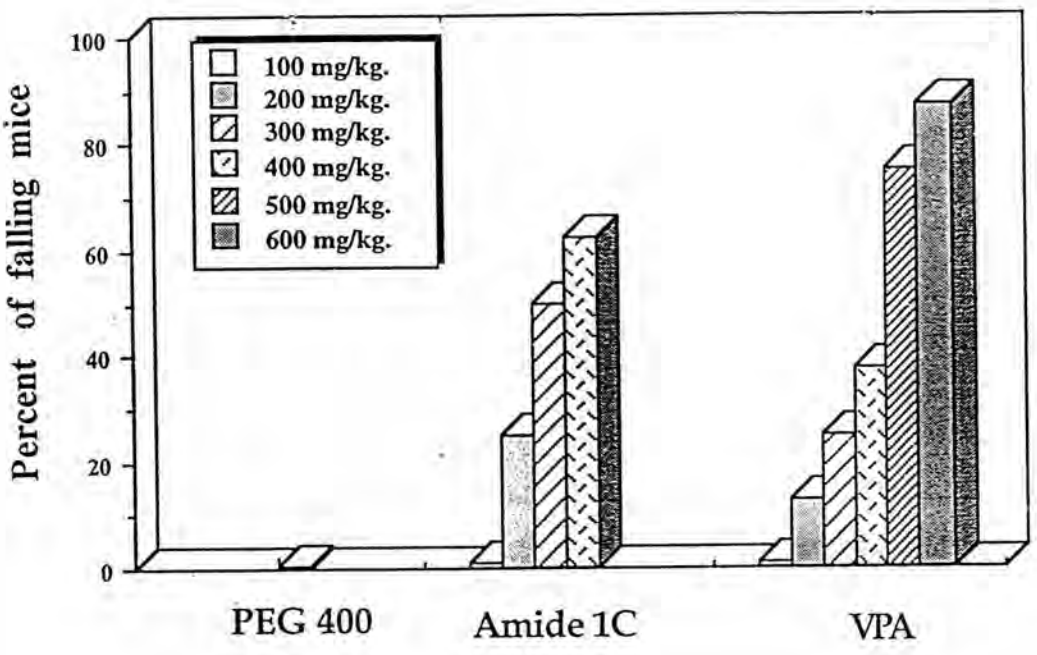
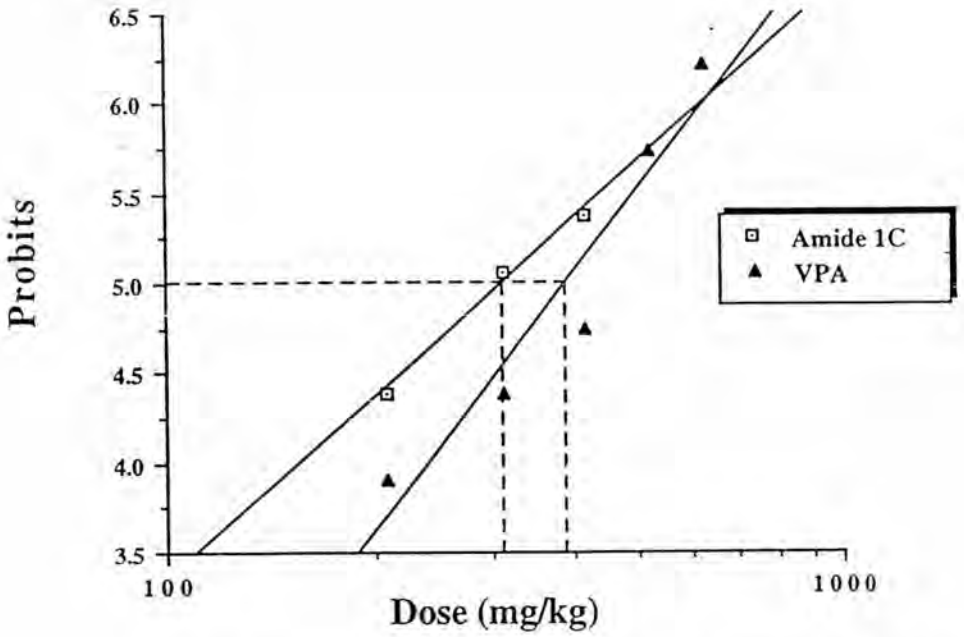


Figure 13. Comparative neurotoxicity (%) of vehicle (PEG 400) and test substances, Amide 1C and VPA, given intraperitoneally in mice.



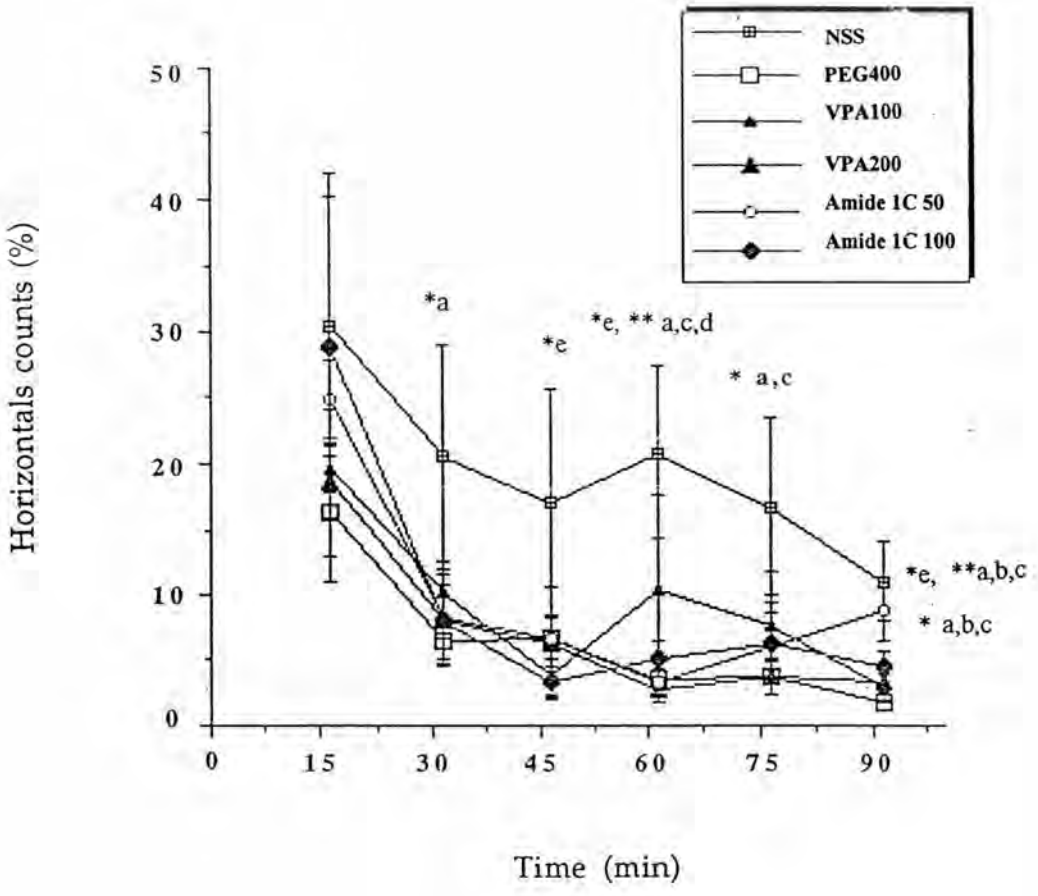
Probits (Amide 1C) = -3.3202 + 3.3339 *LOG(x), R^2 = 0 .989
TD_{50} = 313 (219-441) mg/kg.

Probits (VPA) = -7.5403 + 4.8519*LOG(x), R^2 = 0 .999
TD_{50} = 386 (297-502) mg/kg.

Figure 14. Log dose-response curves of Amide 1C and VPA in rotorod test in mice.

Parameters (mg/kg.B.W.)	Tests	Substances	
		Amide 1C	VPA
TD_{50}	Rotorod	313	386
LD_{50}	-	602	832
ED_{50}	MES	81	218
	PTZ	33	93
PI (TD_{50}/ED_{50})	MES	3.86	1.77
	PTZ	9.48	4.15
Relative Safety Margin (LD_{50}/ED_{50})	MES	7.43	3.82
	PTZ	18.24	8.95

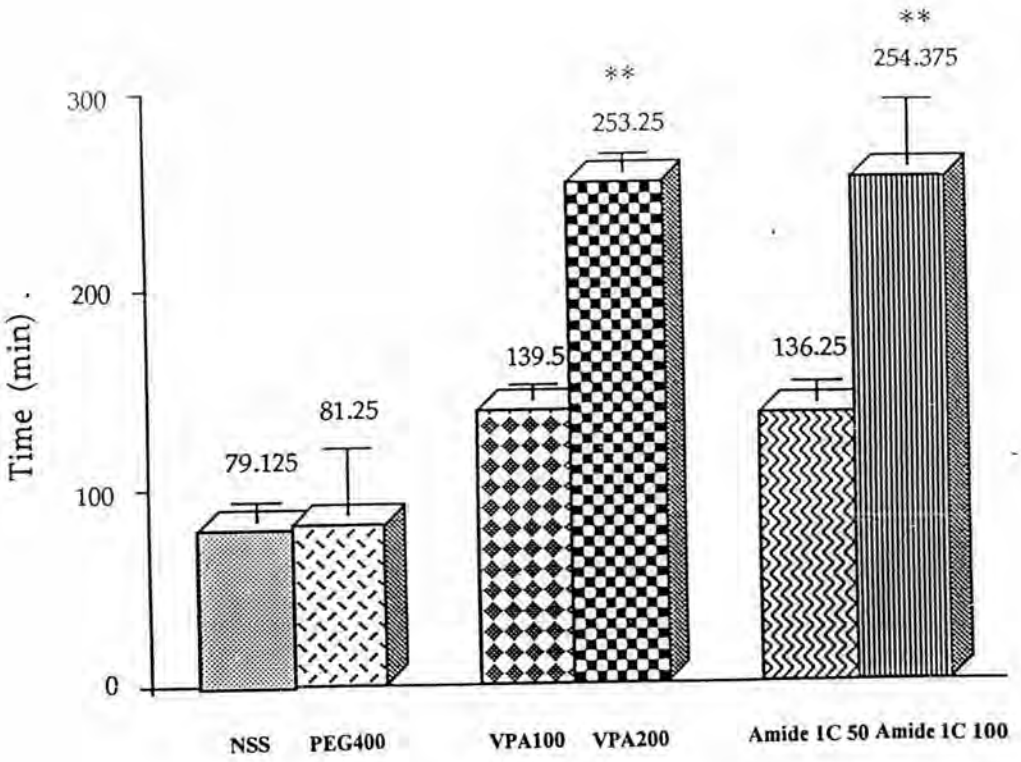
Table 4. ED_{50},TD_{50}, LD_{50} , PI and relative safety margin of intraperitoneal administrations of Amide 1C and VPA in MES and PTZ seizure tests in mice.



a, denotes statistially significant from PEG400
b, denotes statistially significant from VPA100
c, denotes statistially significant from VPA200
d, denotes statistially significant from AMIDE 1C
e, denotes statistially significant from AMIDE 1C

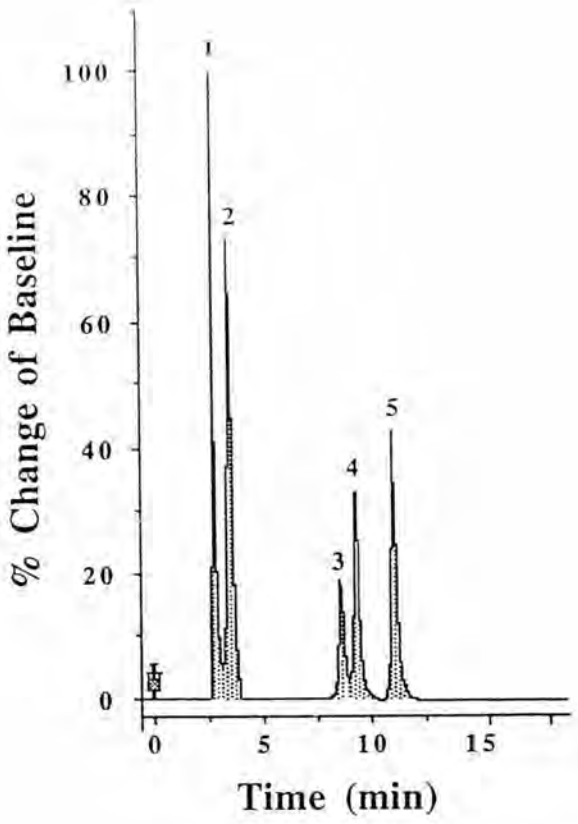
* $P < 0.05$
** $P < 0.05$

Figure 15. Effects of intraperitoneal administration of Amide 1C and VPA on horizontal counts (Mean ±S.E.M.) of locomotor activity in mice at various times.



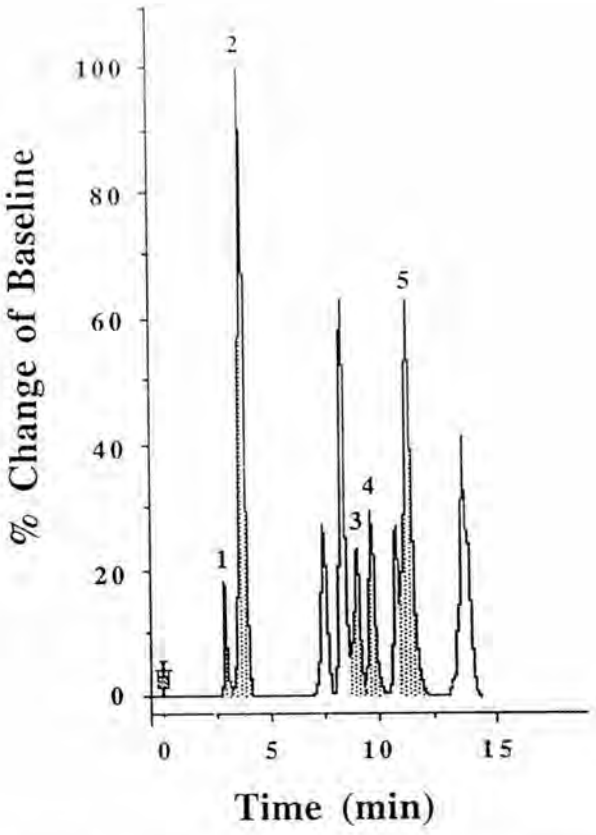
** $p < 0.01$ denote statistically significant from NSS, PEG400, VPA100and AMIDE 1C 50

Figure 16. Effects of intraperitoneal administration of Amide 1C and VPA on the barbiturate sleeping time (Mean ±S.E.M.) in mice.



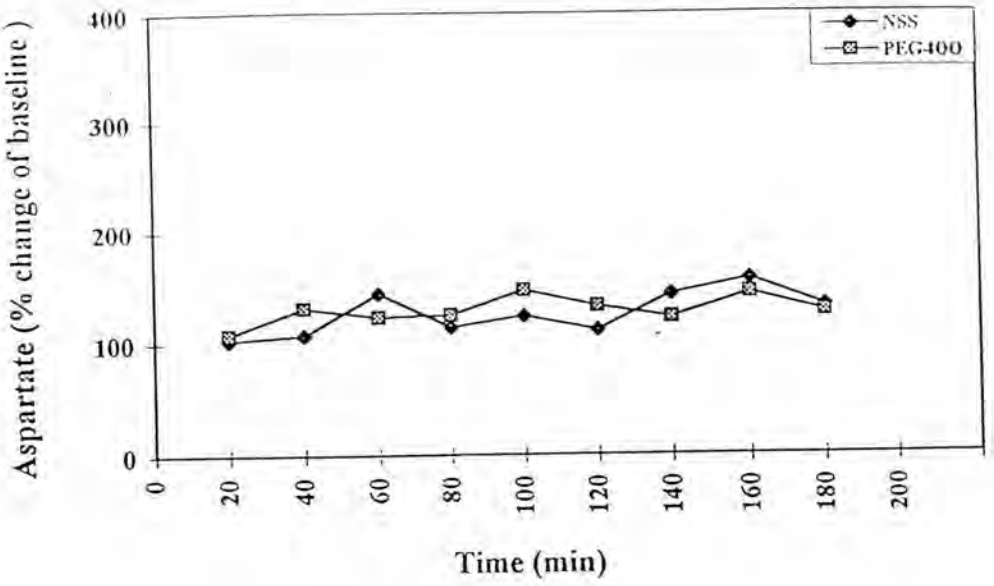
Peak	Name	t_R (min)	Start (min)	End (min)	Area	Height (%)	Norm (%)
1	Aspartate	3.25	2.95	3.60	15.878	100.000	24.55
2	Glutamate	3.95	3.60	5.10	20.938	73.126	32.38
3	Homoserine	9.05	8.25	9.45	5.609	18.749	8.67
4	Glycine	9.75	9.45	10.65	10.142	32.902	15.68
5	GABA	11.55	11.20	12.35	12.102	42.509	18.71
					64.67	267.286	100.00

Figure 17. HPLC chromatogram of OPA-derivatized standard amino acids.



Peak	Name	t_R (min)	Start (min)	End (min)	Area	Height (%)	Norm (%)
1	Aspartate	3.35	2.15	3.70	3.57	18.246	4.53
2	Glutamate	4.15	3.70	5.60	31.96	100.000	40.53
3	Homoserine	9.50	9.15	9.85	8.033	23.100	10.19
4	Glycine	10.15	9.85	10.90	9.548	28.727	12.11
5	GABA	11.80	11.45	12.80	25.735	61.252	32.64
					78.85	231.326	100.00

Figure 18. HPLC chromatogram of OPA-derivatized amino acids from the rat cerebral cortex



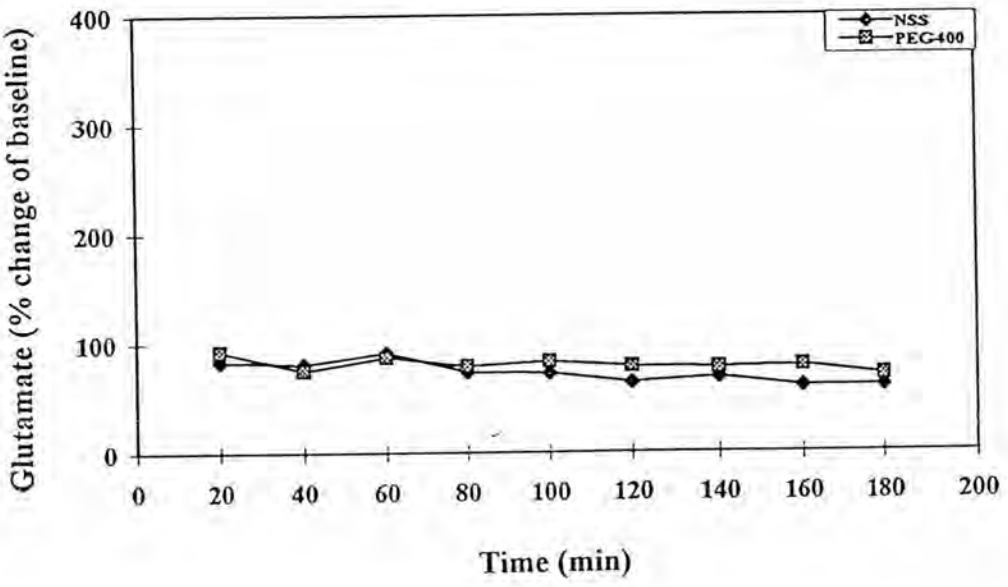


Figure 19. Changes in the rat cortical aspartate and glutamate levels at various times after intraperitoneal administration of NSS and PEG 400.

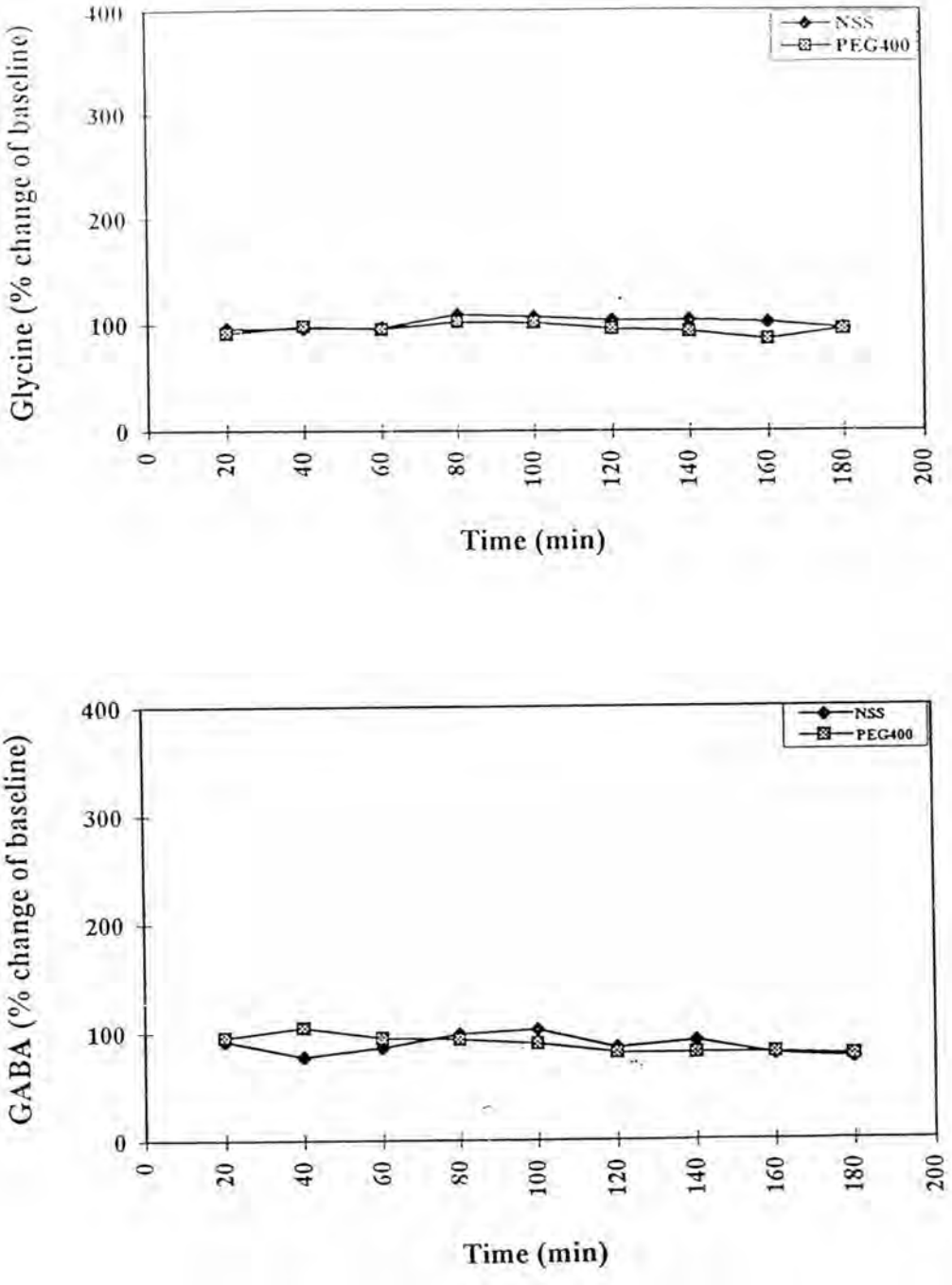


Figure 20. Changes in the rat cortical glycine and GABA levels at various times after intraperitoneal administration of NSS and PEG 400.

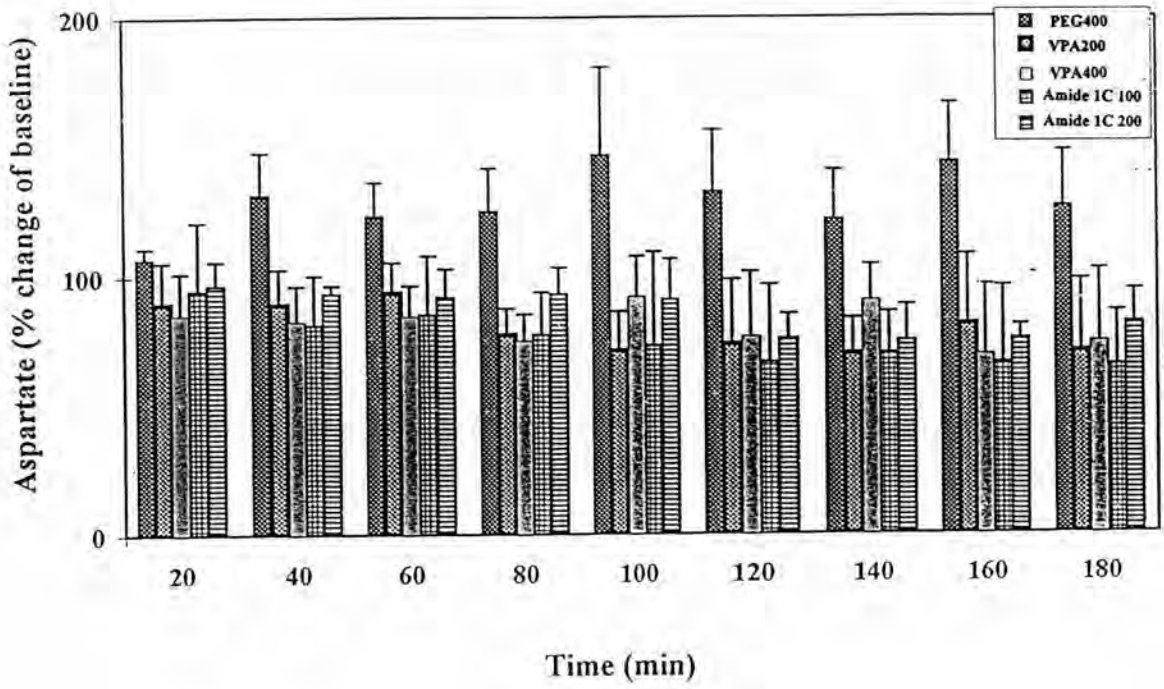


Figure 21. Effects of intraperitoneal administration of Amide 1C and VPA on the rat cortical aspartate levels at various times.

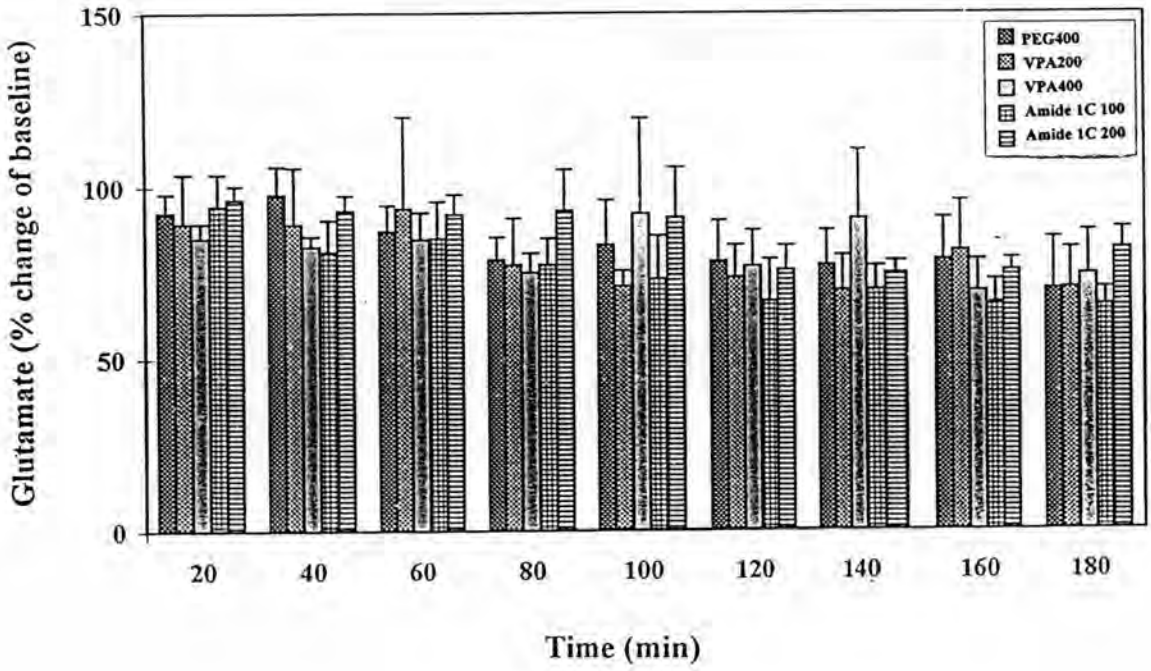


Figure 22. Effects of intraperitoneal administration of Amide 1C and VPA on the rat cortical glutamate levels at various times.

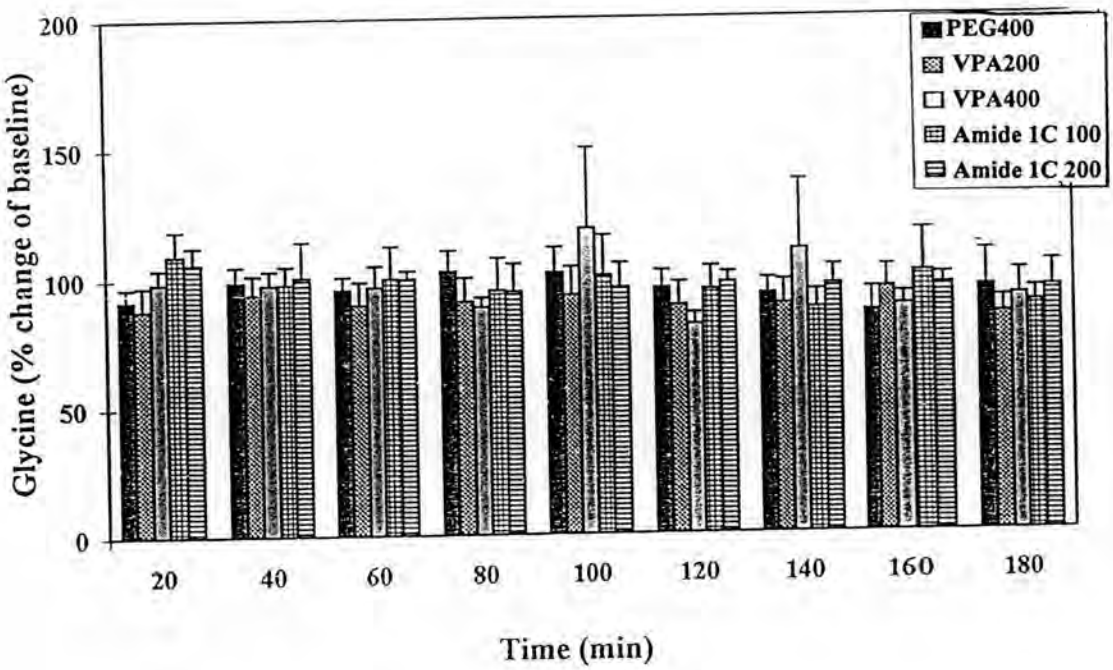


Figure 23. Effects of intraperitoneal administration of Amide 1C and VPA on the rat cortical glycine levels at various times.

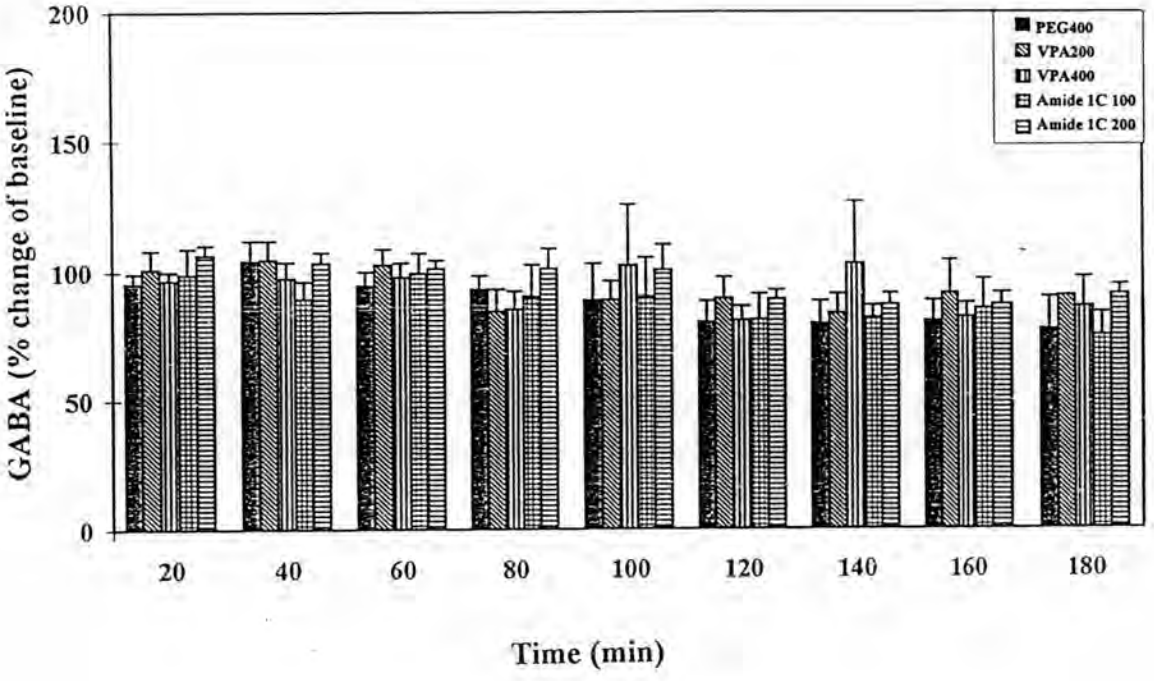


Figure 24. Effects of intraperitoneal administration of Amide 1C and VPA on the rat cortical GABA levels at various times.