Chapter III

Results

Part A : Iontophoresis

Tests were made on spontaneously active neurones located in cerebellar cortex. In agreement with previous report (Tongroach et al. 1984), Purkinje cell (P cell) was depressed by electrophoretic administration of Angiotensin II (AII). Table 2 shows summary of iontophoretic study of AII on cortical neurones of the cerebellum.

Response of Purkinje Neurones to AII Administration

Satisfactory unitary recording were obtained from 45 cerebellar cortical neurones which were identified as Purkinje cells.

In 38 of 45 vermian Purkinje cells (84.44%), the effects of iontophoretic application of AII (20-100 nA, positive current) were consistent depression of the spontaneous discharge in a dose-dependent manner. The response were rapid in onset, and immediate recovery was

observed upon cessation of AII ejection. These inhibitory effects were noticable from iontophoretic current as low as 20 nA, with high ejecting currents a complete suppression of Purkinje cell discharge were attended (Tongroach et al. 1984). For example, Figure 11 A show the development of inhibition of neuronal firing of a Purkinje cell in the vermis during the application of AII 70 nA, 80 nA and 100 nA respectively

Table 2 Summary of iontophoretic study of AII on cortical neurones of the cerebellum, excited (+), ingibited (-) or potentiated inhibition (=)

	Number of Neurones	
	Tested	Responsive
a) Depressant action of AII		
1) Purkinje cells of vermis	45	38
2) Non Purkinje cells	7	0
b) AOAA induced enhancement of the actions		
1. AII	38(-)	29(=)
2. GABA	36(-)	27(=)
3. glycine	29(-)	2(=)
4. glutamate	3(+)	0
5. aspartate	3(+)	0
c) Antagonism of the actions by Saralasin		
1. AII	3(-)	3
2. GABA	3(-)	0
3. GLY	3(-)	0
D) Antagonism of the actions by saralasin on AOAA induced potentiation (5 cells)		
1. AII	5(=)	5
2. GABA	5(=)	0

Effects of Iontophoretic Application of AII on Unidentified Cortical Neurones (Non-Purkinje Neurones)

In contrast, in 7 unidentified non-Purkinje cells, iontophoretic application of AII with currents upto 150 nA failed to produce any changes in the neuronal firing (Figure 11 B)

Effects of Iontophoretic Application of Amino-oxyacetic acid (AOAA)

Amino-oxyacetic acid ($HOOCCH_2ONH_2$), has been reported as inhibitor of GABA-keto-glutaric transaminase (GABA-T) and glutamic acid decarboxylase (GAD), the GABA biosynthesizing enzyme. Administration of AOAA in vivo resulted in an increase in brain GABA concentrations, the elevation of GABA levels induced by AOAA being the greatest and more sustained (Metcalf, 1979). In these experiment, AOAA was used to determine whether iontophoretically administration of AOAA induced enhancement of the depressant action by AII and GABA.

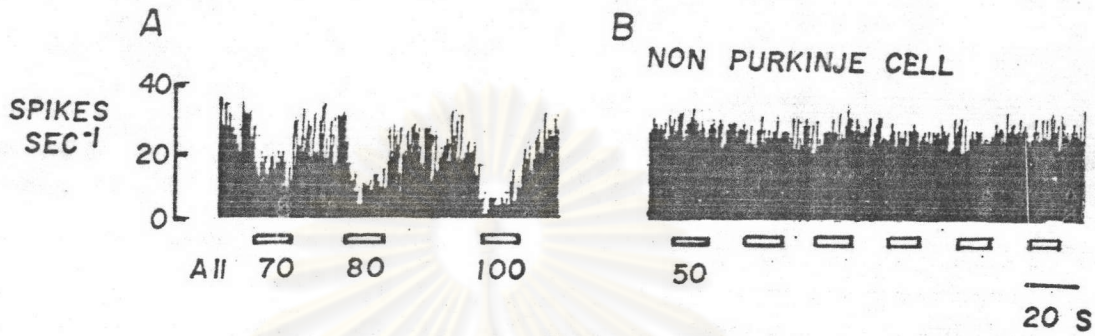


Figure 11 A and B Effects of iontophoretic administration of Angiotensin II (AII) on spontaneous firing of cerebellar neurone. AII produced firing depression on Purkinje cell in a dose dependent (A), where as in B, unidentified non-Purkinje cells did not response to the peptide. In This and the proceeding figures. Firing rates of the neurone in spike per second are shown in vertical axis, horizontal bars underneath or above the records show the periods of iontophoretic application, while figure accompanying each bar indicater iontophoretic current in nA. Time scales is shown as bar at the lower right of the figure and is identifical in both A and B.

Effect on the response to iontophoretically applied AII with superposition of AOAA

In Purkinje cells (n=38) which were consistently depressed by iontophoretically administered AII (20-80 nA) superimpostion of AOAA (10-40 nA) was made over pulsatile application of AII in order to assess any possible enhancement of the actions of the peptide. Of 38 cells tested, AOAA induced enhancement of the depressant effect of AII in 29 cells (76.32%) within 0.5-4 min., the remainder being unaffected. Example of this results is shown in Figure 12 in which it was shown that pulsatile iontophoretic application of AII (75 nA at 10 sec. periods) produced consistent decrease in Purkinje cell discharge. During continuous AOAA application (25 nA), the AII induced depression of spontaneous discharge was remarkably potentiated. Recovery to the control level of response was observed in each case after cessation of AOAA administration.

Response of Purkinje cells to iontophoretically applied AII, GABA and GLY: Selective Potentiation of AII and GABA induced inhibition by AOAA

Effects on the responses to iontophoretically applied depressant amino acids (GABA and Glycine) and AII.

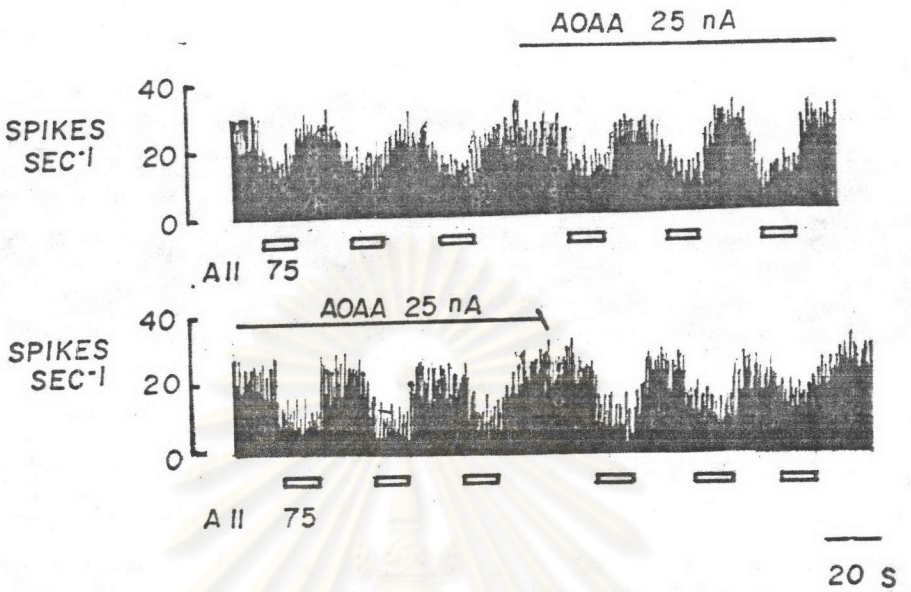


Figure 12 Effect of microiontophoretic administration of AOAA (25 nA) on response of Purkinje cell to microiontophoretic AII (75 nA). It was shown that pulsatile iontophoretic application of AII at 10 sec periods produced consistent decrease in Purkinje cell discharge. During continuous AOAA application, the AII induced depression of spontaneous discharge. Recovery to the control level of response was observed in each case after cessation of AOAA administration.

Pulsatile cationic iontophoretic application (10 sec duration) of putative inhibitory amino acid neurotransmitters (GABA,Glycine) and AII. GABA (10-40 nA, n=36), Gly (40-100 nA, n=29) and AII (20-80 nA, n=38) produced consistant depression of Purkinje cells tested. When continuous microiontophoretic application of AOAA (10-40 nA) were superimposed on the pulses of putative neurotransmitters and AII, the inhibitory response to GABA (27 of 36 cells) and AII (29 of 38 cells) were selectively augmented during the period of AOAA application, (Potentiation of Gly) induced depression although observed was not reproducible (2 of 29 cells). Figure 13 shows that application of AII and GABA, without AOAA application, produced approximately 40 percent depression of spontaneous discharge while during application of AOAA, these depressants induced almost complete suppression of Purkinje cell firing. Note in this case that recovery of the potentiation was observed. Another example is also presented in Figure 14 in which it is shown that pulsatile iontophoretic applications of GABA (25 nA, 10 sec), Gly (70 nA, 10 sec), and AII (75 nA, 10 sec) produced approximately 50 percent depression of spontaneous discharge. When continuous microiontophoretic application of AOAA (25 nA) was superimposed on depression pulses, the inhibitory

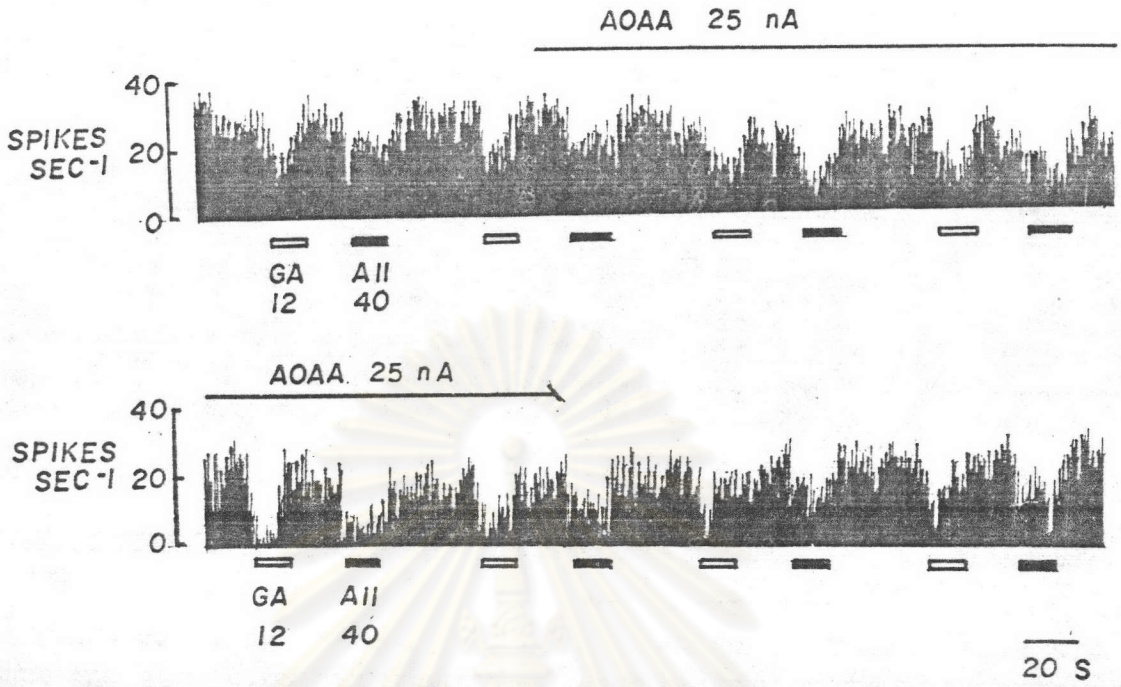


Figure 13 Effect of microiontophoretic administration of AOAA (25 nA) on responses of a Purkinje cell to iontophoretic application GABA (GA12 nA) and AII (40 nA). The rate meter shows that AOAA induced almost completely suppression of Purkinje cell firing during pulsatile applications of GABA and AII. Recovery of the potentiation to the control level was observed after cessation of AOAA administration.

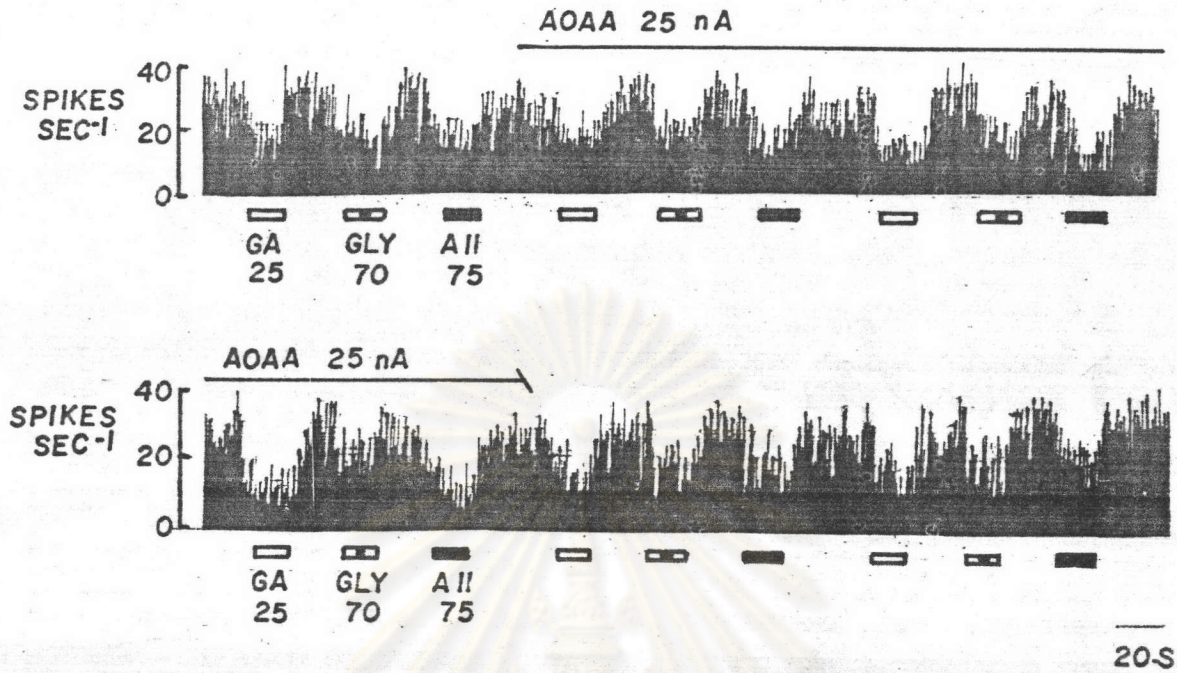


Figure 14 Effect of microiontophoretic administration of AOAA (25 nA) on responses of a Purkinje cell to iontophoretic application GABA (GA, 25 nA), GLY(70 nA). The rate meter shows that AOAA potentiated depressant action of GABA and AII, whereas the action of GLy remained uneffected. Recovery to the control level of response was observed after cessation of AOAA administration.

response to GABA and AII were potentiated, while pulsatile application of Gly which produced depression of the spontaneous discharge remained almost uneffected. The onset of AOAA induced potentiation of GABA response was apparent within 0.5-4 min. after commencement of AOAA application and this potentiation was progressive with time. The iontophoretic currents of AOAA in this case were adjusted to be subthreshold for producing depression of each neurone tested, so that depression induced by AOAA was considered minimal. After the termination of AOAA application, recovery to the control level of responses was observed in each case.

Effects on the response to iontophoretically applied excitant amino acids (glutamate and aspartate)

Tests were also advanced further to see whether there was any AOAA-induced modification of excitatory responses of Purkinje cell to putative excitatory neurotransmittes, glutamate (GLU) and aspartate (Asp). Pulsatile iontophoretic application (10 sec duration) of Asp (25-40 nA, n=3) and Glu (20-40 nA, n=3) produced consistent excitation in all Purkinje cells tested. When continuous microiontophoretic application of AOAA (25-40 nA) were superimposed on the excitation pulses,

the excitation of Purkinje cells activity (3 cells) caused by Asp and Glu remained uneffected., Example of these results is shown in figure 15.

Effects of Iontophoretic Application of Saralasin, an Angiotensin II Antagonist

Saralasin, a specific AII antagonist, is a synthetic peptide which has the same structure as AII except that it is substituted in the 1 position by sarcosine and in the 8 position by alanine, making it a specific competitive inhibitor of AII. In the course of iontophoresis experiments, Phillip and Felix (1976) showed that the neuronal response to AII was totally inhibited during ejection of saralasin. In these experiments, saralasin was used as a tool to investigate the receptor mechanism of AII involvement in depression of spontaneous Purkinje cell activity.

Effect of Saralasin on the response to iontophoretically applied GABA, GLY and AII

Purkinje cell (n=3) which were consistently depressed by iontophoretically administered of GABA (20-40 nA, n=3), GLY (10-60 nA, n=3) and AII (60-100 nA, n=3) were tested for the antagonistic activity of iontophoretic application of saralasin (10-20 nA). In

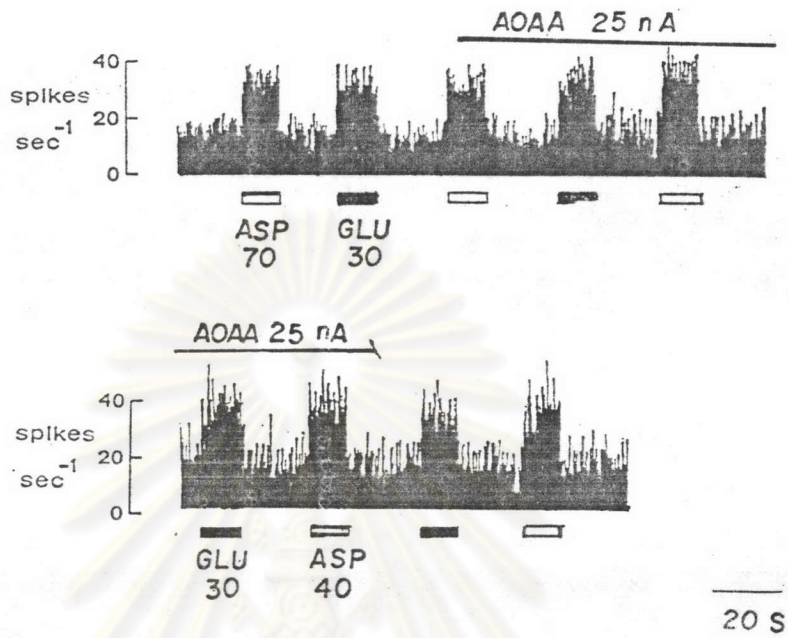


Figure 15 Effect of continuous application of AOAA (25 nA) on excitant action of aspartate (Asp; 70 nA) and glutamate (glu; 30 nA) on Purkinje cell. The rate meter shows that no change in the neuronal responsiveness to the excitants.

all cells tested, saralasin antagonized the depressant effect of AII. Example of these results is shown in figure 16, in which it was shown that pulsatile iontophoretic application of AII (75 nA, at 10 sec. period) produced consistent decrease in Purkinje cell discharge. During continuous saralasin application (10 nA), the AII induced depression of spontaneous discharge was antagonized, while the depression of neuronal activity caused by the control agonists GABA and GLY remained uneffected. Recovery to the control level of response was observed in each case after cessation of saralasin administration.

Effect of Angiotensin II antagonist (Saralasin) on AOAA Induced potentiation of actions of GABA, GLY, and AII

In Purkinje neurones (n=5) which were consistently depressed by pulsatile iontophoretic application (10 sec. duration) of GABA (20-50 nA, n=5), GLY (60-100 nA, n=5) and AII (60-80 nA, n=5), continuous iontophoretic applications of AOAA (25-50 nA) produced enhancement of GABA and AII in inducing firing rate depression in 5 (total n=5) neurones tested without having any effects on action of GLY. When saralasin was superimposed on AOAA application, AOAA induced enhancement of AII were gradually antagonized without

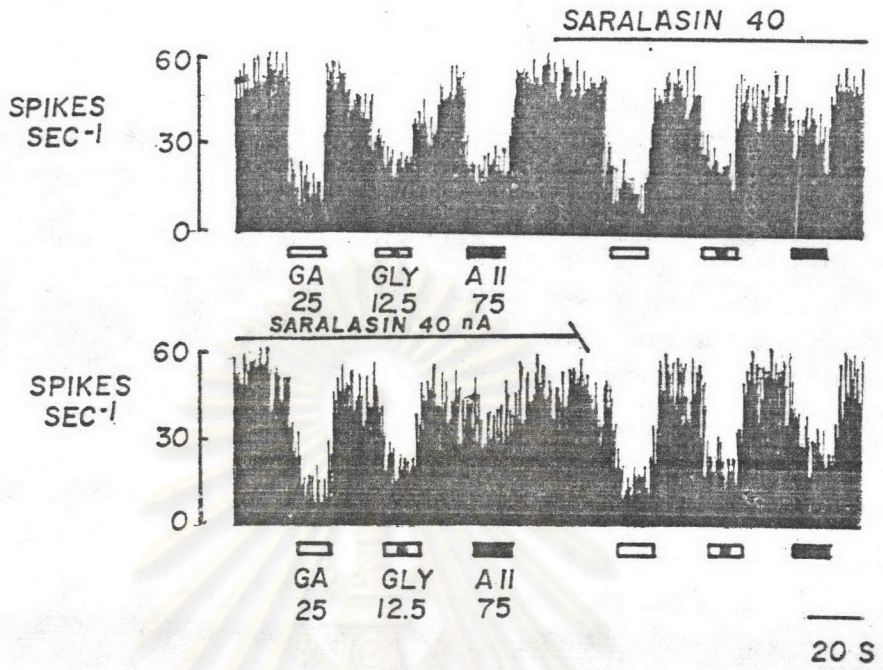


Figure 16 Effect of microiontophoretic application of Angiotensin II antagonist, saralasin (40 nA) on the responses of Purkinje cell to microiontophovtically applied GABA(GA, 25 nA), GLy (12.5 nA), and AII 75 nA. The rate meter shows that saralasin selectively antagonized depressant action of AII, whereas the depresion of neuronal activity caused by GABA and Gly remained uneffected. Recovery to the control level of response was observed in each case after cessation of saralasin administration.

any effects on action of GABA. However, subsequent manifestation of the enhancement under persistant AOAA ejection were also observed after termination of Saralasin application. The recovery from AOAA effect was also observed following cessation of AOAA application. Example of these results is shown in Figure 17.

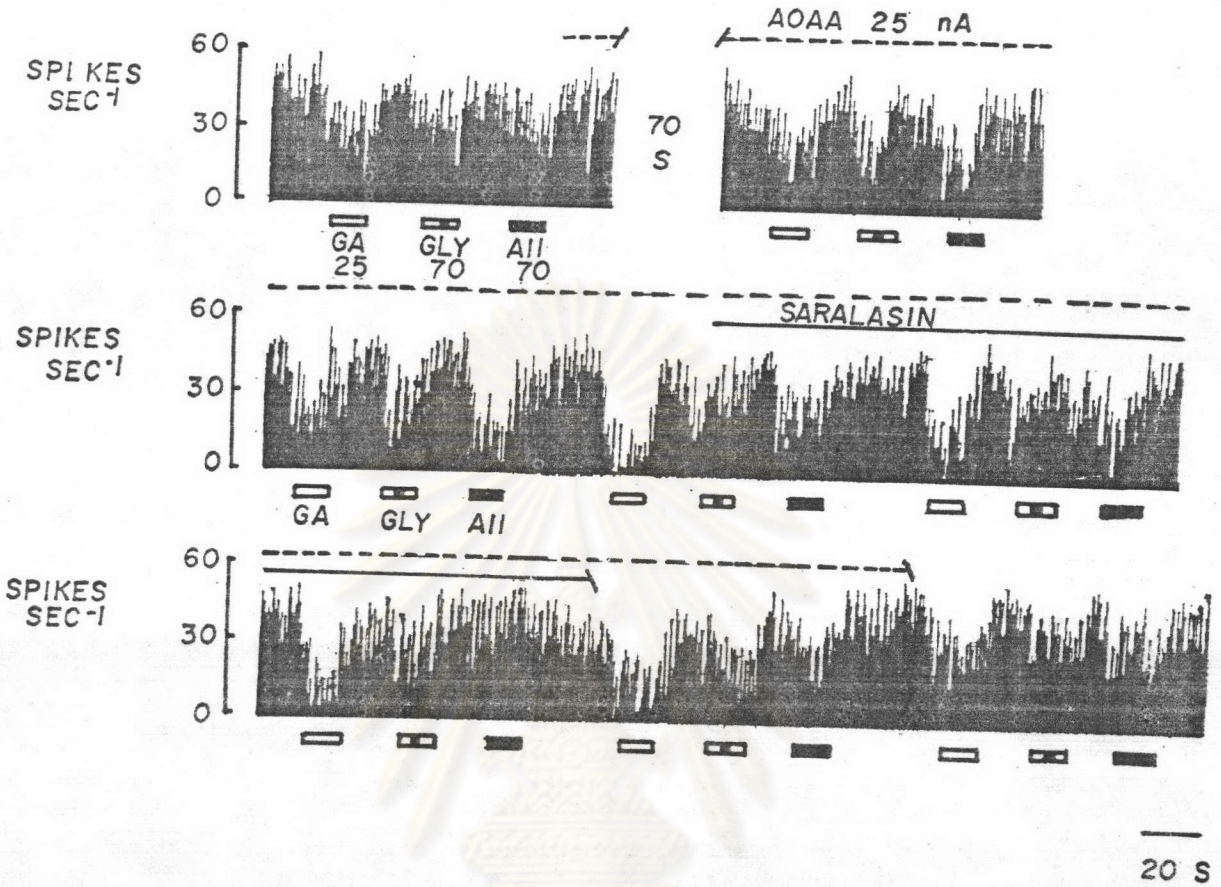


Figure 17 Effect of AII antagonist, saralasin (40 nA) on AOAA induced potentiation of actions of GABA and AII. The rate meter shows that continuous application of AOAA (25 nA) produced enhancement of depression action of GABA (25 nA) and AII (70 nA) on Purkinge cell discharge while the neuronal activity caused by GLy (70 nA) remained unaffected. When saralasin (40 nA) was superimposed on AOAA application, AOAA induced enhancement of AII were gradually antagonized, without any effects on action of GABA. However subsequent manifestation of the enhancement was observed after termination of saralasin application. Recovery to control was observed following cessation of AOAA application.

Part B: Biochemical results

The results were obtained from perfusates of the rat cerebellar cortex. Collected by push pull perfusion, High performance liquid chromatography (HPLC) was used for the quantitative detection of amino acids in the perfusates.

Amimo Acid Analysis : Results with amino acid standards

Under the chromatographic conditions described, a typical separation of OPA derivatives of a standard mixture of 8 selected amino acids has been acheived successfully. Figure 18 shows a typical chromatogram of 8 amino acids (Conc. 400 pmole of each) : aspartic acid (Asp), glutamic acid (GLu), serine (Ser), glutanime (Glun) , glycine (Gly), taurine (Tau), alanine (Ala) and gamma-aminobutyric acids (GABA). The complete elution of the amino acids was achieved within 20 min. The area of each peak of the chromatogram was directly proportional to the amount of the OPA - derivatives of each standard with linearity realiable in the range 10 pmole to 1600 pmole aminao cid content. Standard curves are presented in Fig 19A and 19B

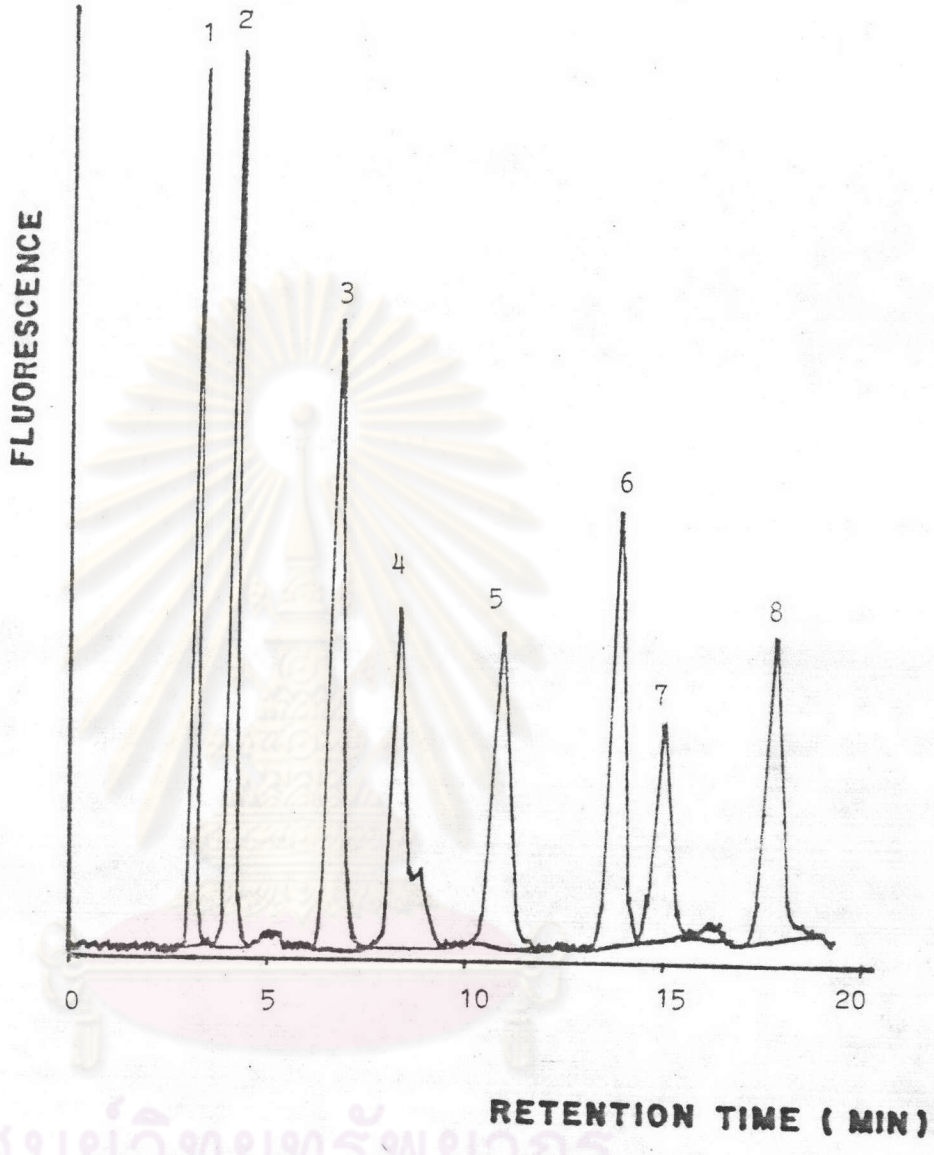


Figure 18 Chromatogram of OPA - derivatives of standard amino acids (400 pmol of each). Peak : 1 = aspartic acid ; 2 = glutamic acid ; 3 = serine ; 4 =glutamine; 5 = glycine; 6. taurine; 7. alanine; 8. GABA.

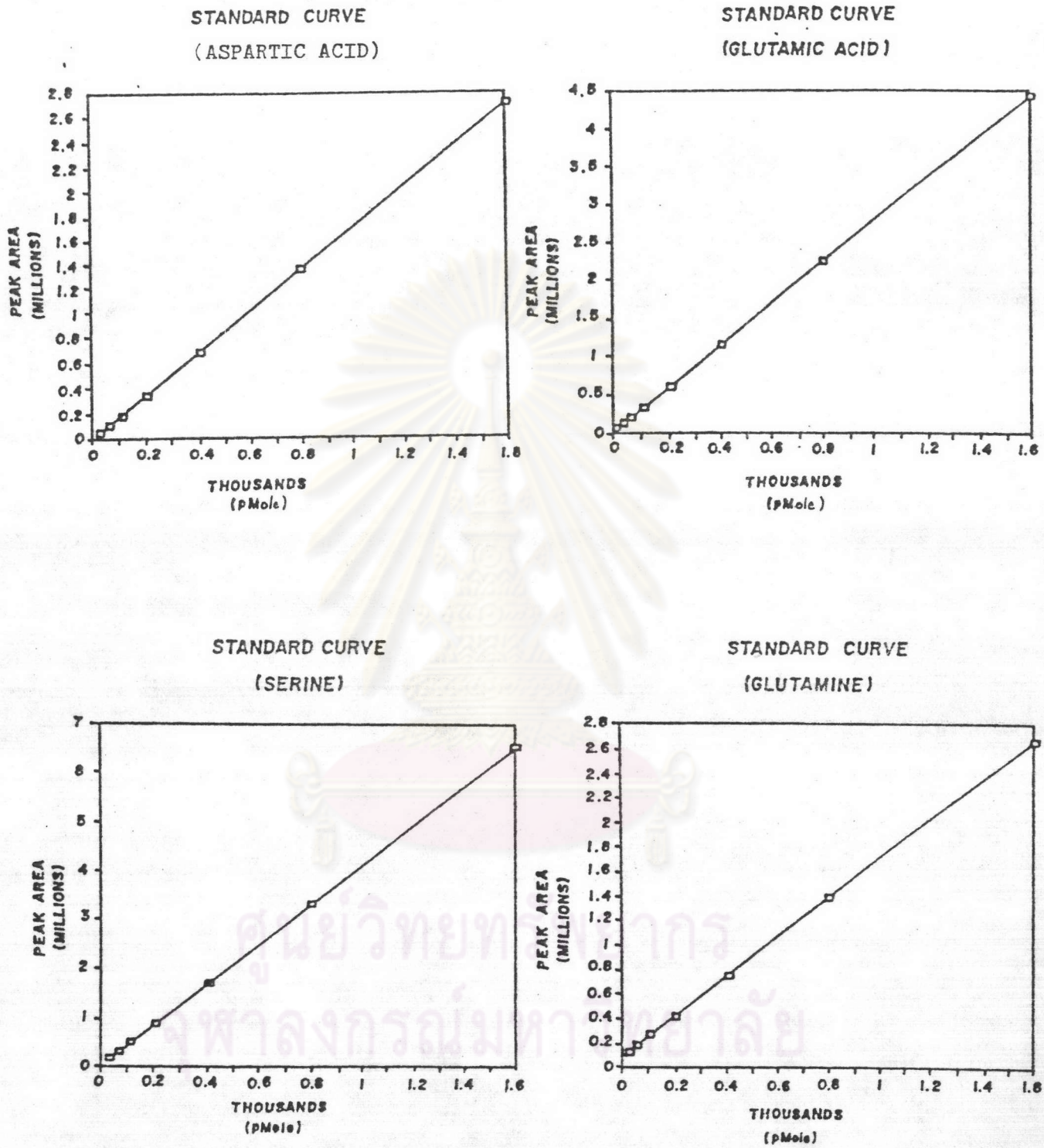


Figure 19 A Standard curve of amino acids measurement (aspartic acid), glutamic acid, serine, and glutamine). Peak area refers to area under each of amino acids peak.

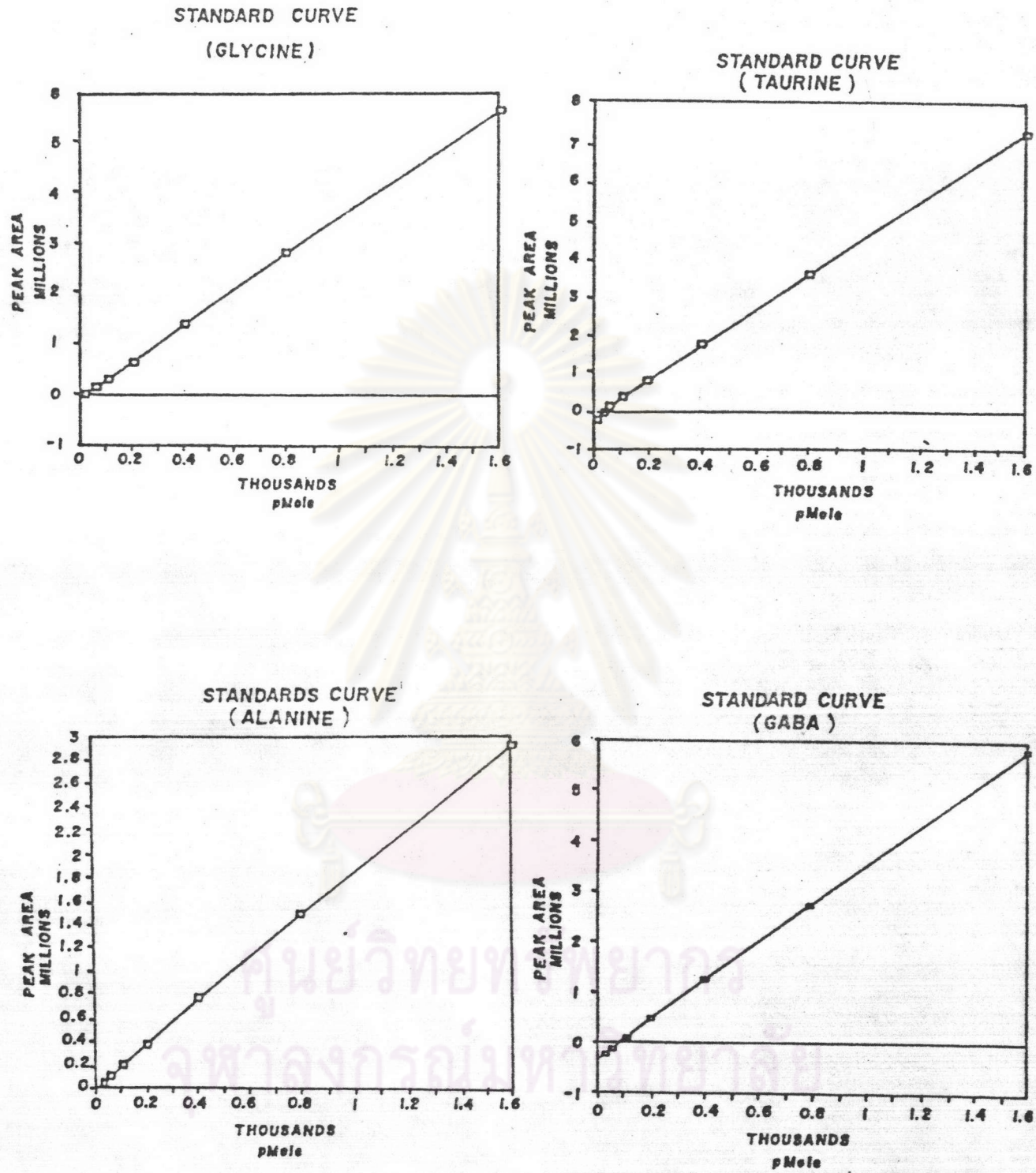


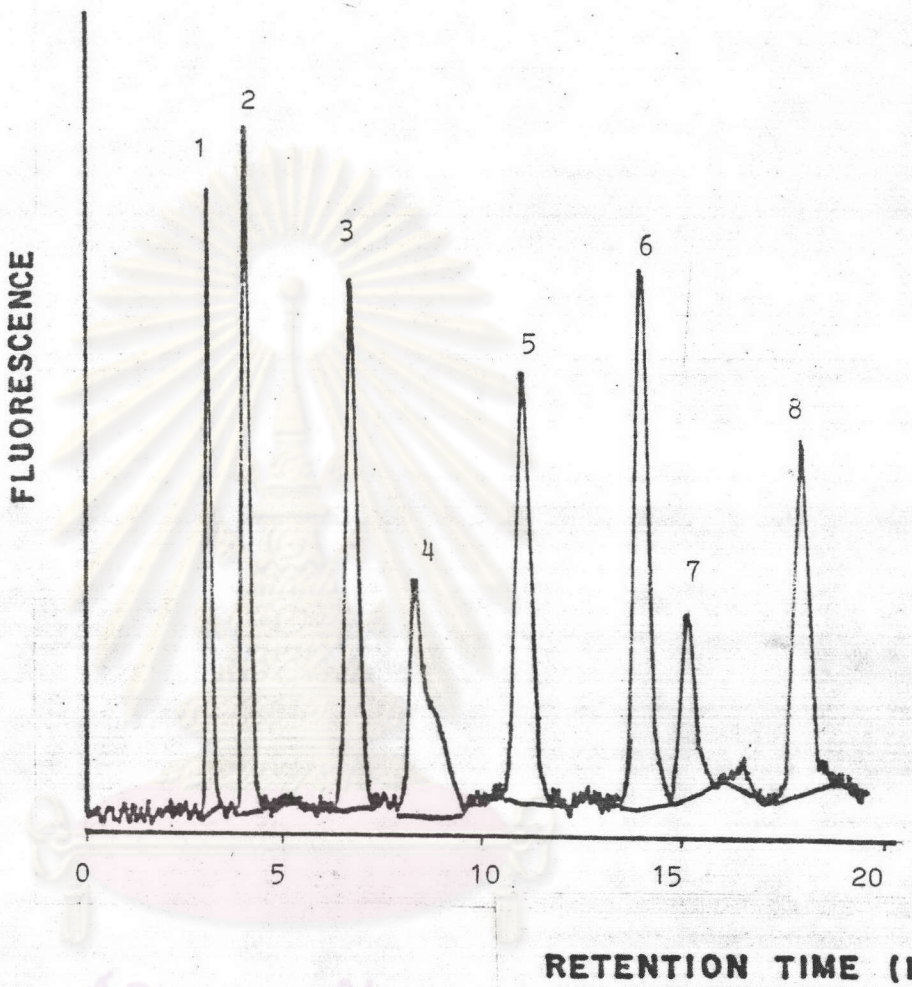
Figure 19 B Standard curve of amino acids measurement (glycine , Taurine, alanine, and GABA). Peak are refers to are under each of amino acids peak.

Perfusion Experiments

The amount of animo acids released into the perfusing medium was strictly dependent on the placement of the push - pull canula. When measurable amounts of amino acids were obtained in chromatographic determination , post - experiment histological examination always revealed correct localization of the canula tip within the cerebellar cortex, as suggested by the tissue damaged on the histological sections. Figure 20 shows an example of chromatogram of perfusate obtained from a successful experiment (figure 21). By contrast, incorrect placement of the canula yielded less conspicuous amount of amino acid release. An example of such case is shown in chromatogram in Fig 22, which was obtained from an incorrect location of the canula (fig. 23)

Spontaneous Release of Endogeneous Amino Acids

When the cerebellar cortex was continuously superfused with standard artificial CSF in a successful experiment, the amounts of various amino acids could be recovered in the perfusate throughout the period of perfusion (110 minutes). The amino acids released, as identified by the corresponding peak numbers in the



1.	aspartic acid	=	467.97	pmol
2.	glutamic acid	=	459.61	pmol
3.	serline	=	416.49	pmol
4.	glutamine	=	809.18	pmol
5.	glycine	=	475.38	pmol
6.	taurine	=	432.14	pmol
7.	alanine	=	421.53	pmol
8.	GABA	=	343.17	pmol

figure 20 Chromatogram of the perfusate sample from the cerebellar cortex. Amount of amino acids shown under the chromatogram were interpretated from standard curve.

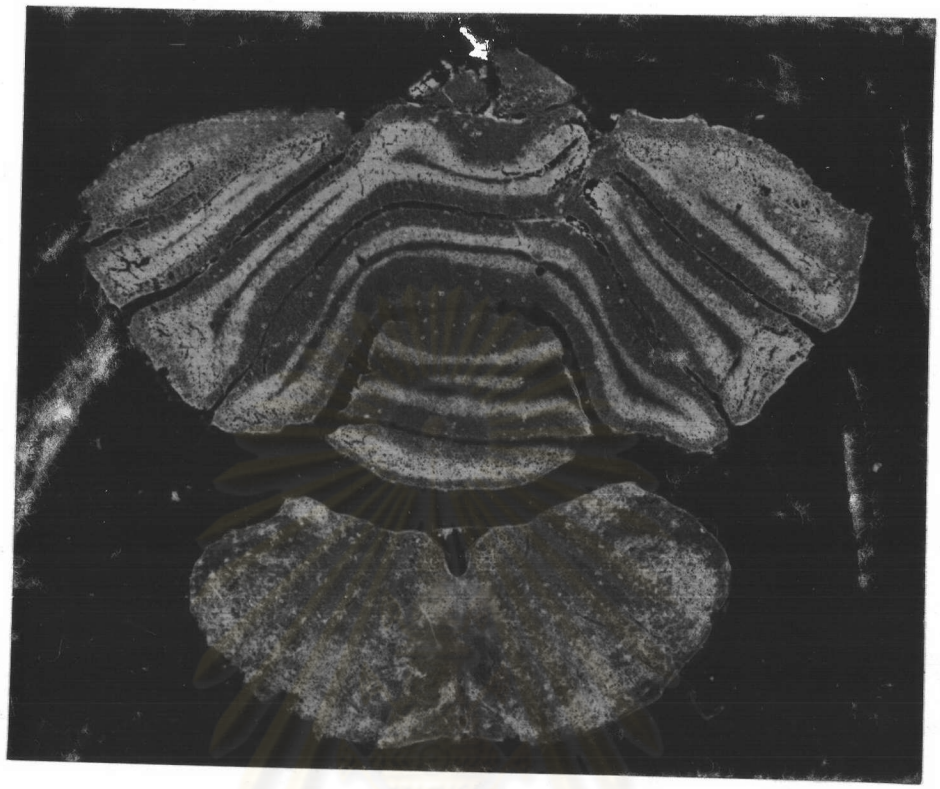


Figure 21 Coronal section of the rat brain from a successful experiment (molecular layer) at the arrow.

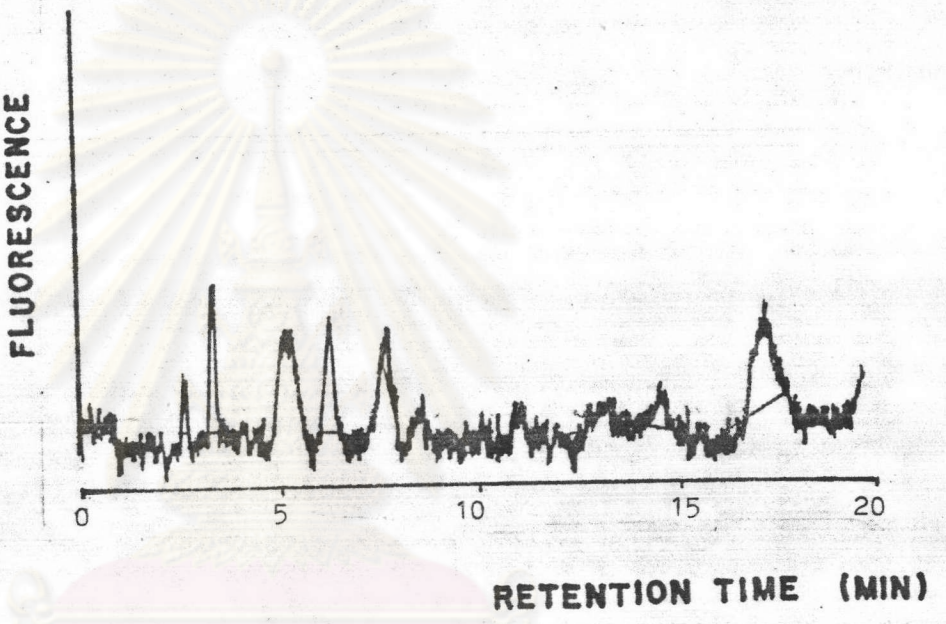


figure 22 Chromatogram of the perfusate sample from incorrect placement of the push - pull canula.

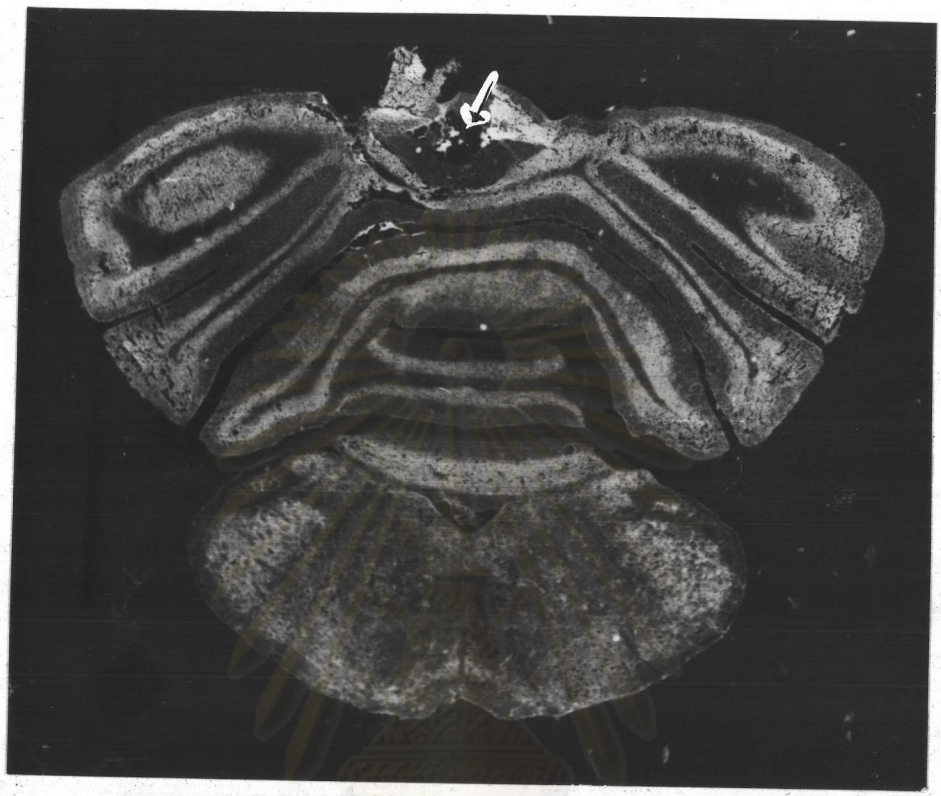


Figure 23 Coronal section of the rat's brain which the canula tip located in incorrect placement (non molecular layer) at the arrow.

chromatogram were Asp., GLu., Ser., GLun., Gly., Tau., Ala., and GABA.

Effect of perfusion with high K^+ solution

An attempt to induce enhanced release of the amino acid was carried out by replacing the artificial CSF with perfusing fluid containing high concentration of K^+, in order to cause local depolarization of the excitable tissue within the vicinity of perfusion.

Once the base line release was established, usually after collection of two spontaneous release, the control artificial CSF was replaced with high K^+ (100 nM) for 20 min,. after that the perfusion was returned to the control solution. Trial exposure to high K^+ (100 nm) significantly increased the release of endogeneous amino acids by 228.1% of Asp. (n=10), 392.69% of GLu. (n=10), 179.3% of Ser. (n=10), 191.25% of GLun. (n=10), 255% of Gly. (n=10), 212.9% of Tau. (n=10), 279.8% Ala. (n=10), and 613.2% of GABA (n=10) (table 3 and Fig. 24A and B and Fig.27)

Effect of Angiotensin II on Amino Acid release

In order to investigation the effects of AII on

	Control		100 mM-K^+		Control			100 mM-K^+		Control	
amino acid \ sample number	1	2	3	4	5	6	7	8	9	10	11
Asp	29.11±6.88	24.34±8.65	61.56±18.96 *	84.19±36.65	33.52±9.73	17.78±7.7	16.28±6.9	24.83±5.17	29.06±8.92	29.62±14.54	5±3.01
GLu	14.71±3.35	13.38±3.99	37.44±9.67	65.37±22.83	24.21±8.28	12.85±3.14	7.30±3.05	23.23±8.88	64.65±37.24	15.47±4.02	4.17±1.87
Ser	45.45±7.92	33.87±7.67	88.66±27.22	76.74±28.70	56.28±18.91	36.67±11.28	19.77±8.65	45.68±12.69	31.92±9.74	50.07±12.44	24.32±14.05
GLn	101.29±58.08	67.65±25.88	218.22±98.84	187.9±80.91	87.49±34.15	118.45±46.69	33.11±12.27	98.47±32.16	107.65±56.94	274.44±179.85	44.07±20.35
GLy	25.99±4.85	17.89±4.60	44.11±11.05	45.28±14.12	21.02±6.12	17.65±5.05	9.6±5.43	44.43±19.67	47.11±26.5	43.43±21.08	9.64±8.46
Tau	12.48±3.99	10.02±6.75	22.13±5.51 *	25.5±8.58	7.4±2.06	10.72±3.26	2.63±1.31	16.36±5.24	11.73±0.37	20.72±9.92	6.17±4.28
Ala	9.01±4	8.88±4.22	30.03±9.26	41.71±18.7 *	17.12±6.3	11.81±6.06	5.99±4.26	25.14±13.65 *	11.87±4.88	40.23±26.1	0±0
GABA	2.38±1.1	1.21±2.08	9.02±2.05	19.02±5.69	2.36±1.02	3.52±1.79	0.32±0.3	8.57±2.38	10.17±4.81	2.26±0.89	0.14±0.13

Table 3 The release of amino acids from the rat cerebellar cortex, evoked release by stimulated with high K^+(100 mM). The values in the table represent the mean total p-mole of amino acids release per minute ± S.E.of mean (p-mol/min). n=number of observation=10

*Significantly different from that released into the control artificial CSF ($P<0.05$)

Figure 24 A and B Effects of high concentration of K^+ dependent stimulated (100 mM) without AII in artificial CSF on the release of endogeneous amino acids from perfusate of rat cerebellar cortex. The initial superfusion media was control artificial CSF : after sample 2, and 7 were collected the medium is changed to high K^+ dependent (A) After sample 4 and 9 were collected the medium was changed to control artificial CSF (B). An asterisk adjacent to a point indicates a significant difference in statistical analysis (Student's t test, $P<0.05$).

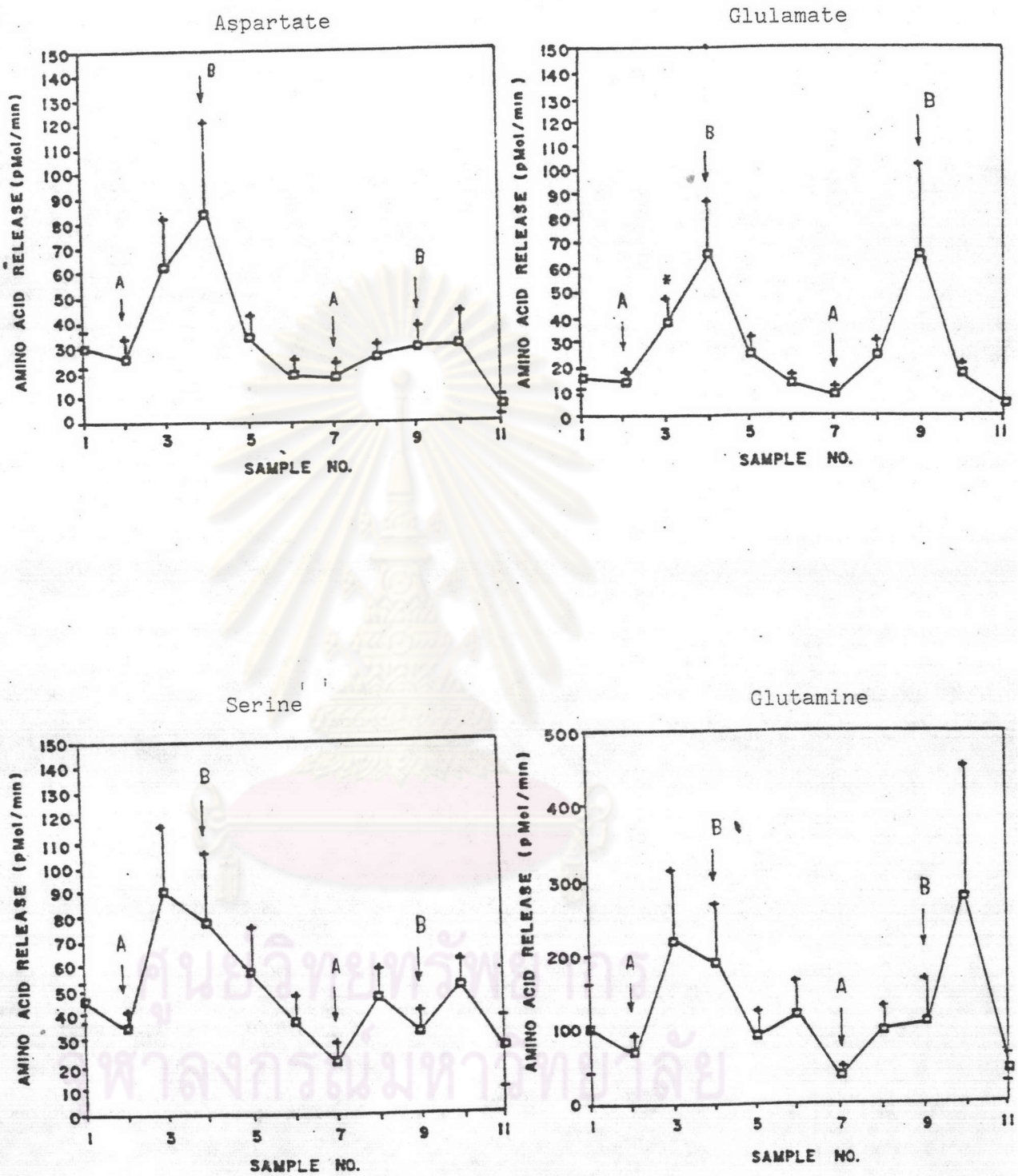

Aspartate
Glulamate
Serine
Glutamine
AMINO ACID RELEASE (pMol/min)
SAMPLE NO.
A
B

Figure 24 A

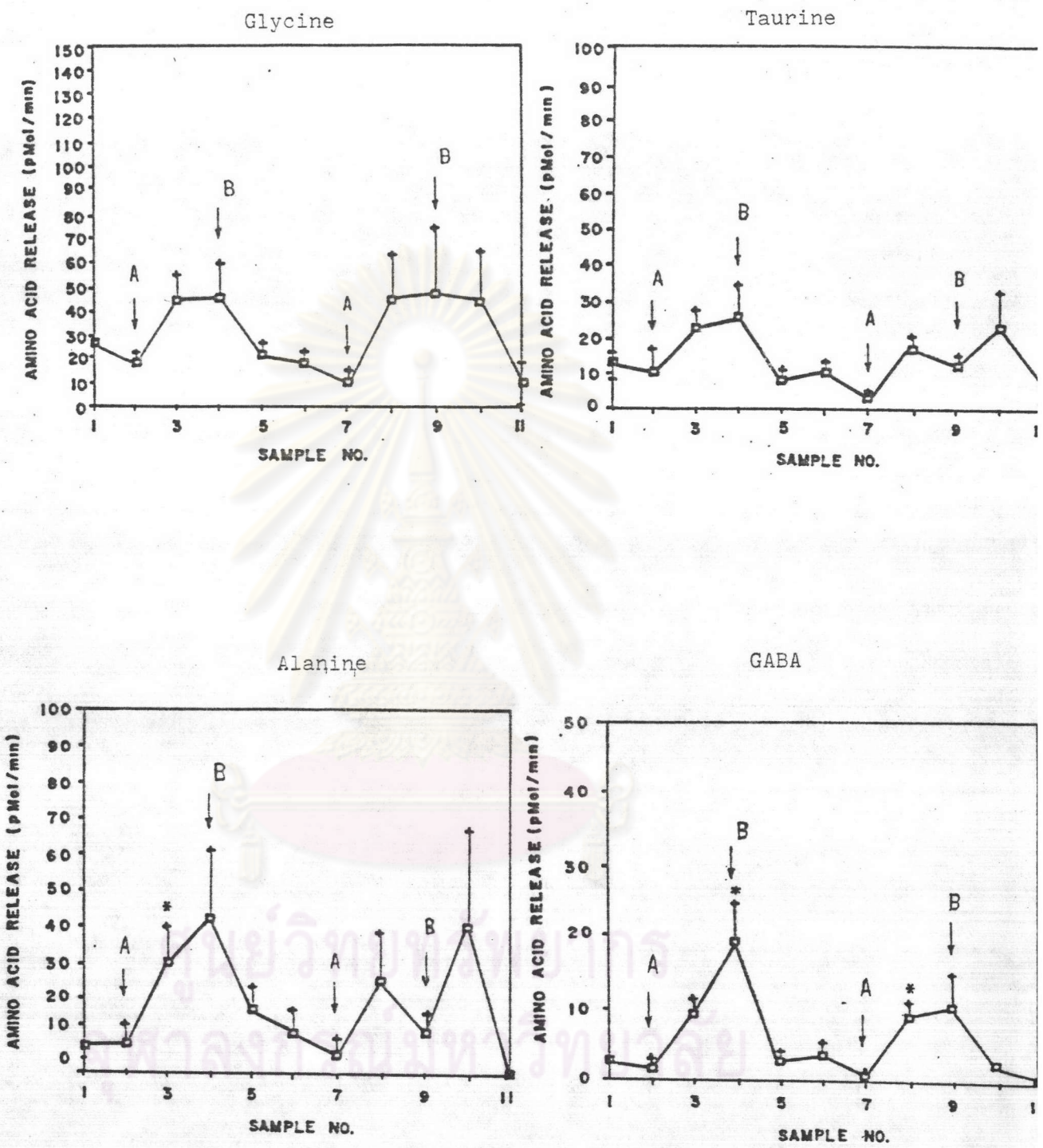
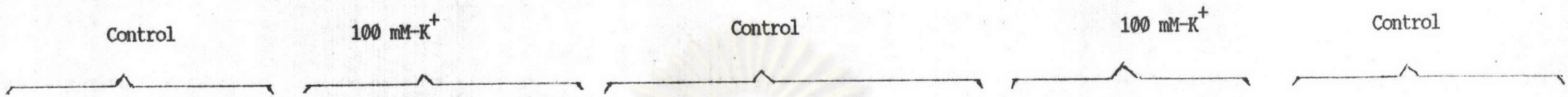

Glycine
Taurine
Alanine
GABA
AMINO ACID RELEASE (pMol/min)
SAMPLE NO.
A
B

Figure 24 B

the release of endogeneous amino acid, experments were carried out by perfusion with artificial CSF containing AII (40 nM). After the control sample collection, the perfusion medium was changed to the high K^+ (100 mM) with AII (40 nM) for 20 min. Then perfusing fluid was replaced with control artificial CSF. In such conditions, AII enhanced the release of endogeneous amino acid, by 290.06% of Asp. (n=6), 256.3% of Glu. (n=6), 178.9% of Ser. (n=6), 273.8% of Glun. (n=6), 290% of Gly. (n=6), 362% of Tau. (n=6), 261% of Ala. (n=6), and 850% of GABA (n=6), Nate the marked increas of GABA release (table 4 and Fig. 25A,B fig.26A,,B,C,D, and fig 27) as compared with cases with AII incorporation.



Amino acid \ sample number	1	2	3	4	5	6	7	8	9	10	11
Asp	34.33±16.36	2.34±1.02	27.49±9.28	76.88±30.41	13.77±5.52	10.65±3.15	17.13±5.41	38.88±27.70	43.56±21.72	28.86±9.85	15.52±12.61
GLu	33.30±13.61	5.49±2.58	20.51±3.99	63.86±20.11	13.21±4.02	9.05±1.48	12.54±5.92	26.46±10.82	43.96±17.69	31.70±13.17	1.35±0.79
Ser	51.90±28.34	37.22±17.49	58.40±15.69	89.09±41.34	34.36±13.84	16.48±4.14	41.17±17.7	71.61±28.55	43.64±12.31	20.69±10.40	6.42±3.25
GLn	81.23±52.16	12.56±8.79	93.51±30.84	118.84±28.89	69.33±10.26	64.69±34.41	21.90±6.48	112.24±37.54	169.38±105.1	62.88±32.52	6.30±2.82
GLy	34.38±16.17	2.13±0.97	19.11±7.62	70.58±39.43	10.86±3.14	7.38±2.90	17.35±7.64	62.75±42.12	27.05±18.62	11.85±5.42	4.87±3.36
Tau	13.88±5.31	2.40±1.78	27.91±16.76	23.04±10.07	9.37±3.74	5.16±3.12	7.98±5.62	17.14±11.60	38.20±15.61	6.17±2.49	0.63±0.57
Ala	24.36±12.73	0.65±0.59	17.27±6.61	35.56±26.43	3.43±2.19	3.23±1.53	6.29±5.73	14.86±9.76 *	23.08±11.64 *	2.00±1.41	0.00±00
GABA	3.31±6.66	2.00±1.82	22.17±9.79	33.80±14.49	4.54±3.02	4.59±3.79	3.14±2.06	25.65±6.65	27.14±6.88	5.56±3.63	0.00±00

Table 4 Evoked release of amino acids from the rat cerebellar cortex by stimulated with high K^+(100 mM) in condition of CSF with AII 40 nM. The values in the table represent the mean total p-mole of amino acid release per minute ± S.E. of mean (p-mole/min). n=number of observation=6

*Significantly different from that released into the control artificial CSF ($P<0.05$)

Figure 25 A and B Effects of high concentration of K^+ dependent stimulated (100 mM) with Angiotensin II artificial CSF on endogeneous amino acids release from The rat cerebellar cortex. The initial superfusion media was control artificial CSF, after sample 2 and 7 were collected the medium is changed to high K^+ dependent (A). After sample 4 and 9 were collected the medium was changed to control artificial CSF (B). An asterisk to a point indicates a significant difference in statistical analysis (Student's t test, P.<0.05>

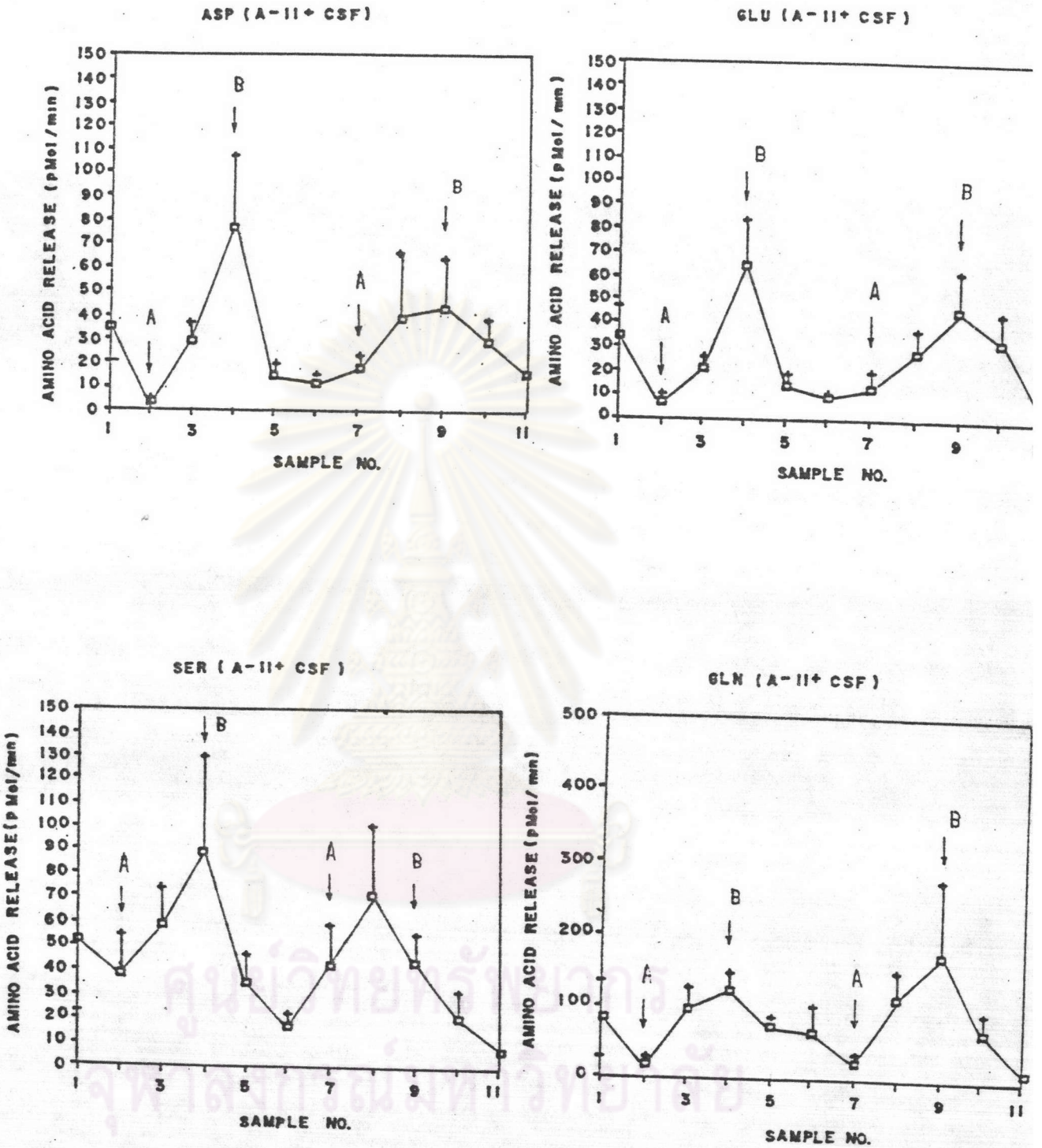

ASP (A-II+ CSF)
GLU (A-II+ CSF)
SER (A-II+ CSF)
GLN (A-II+ CSF)
AMINO ACID RELEASE (pMol/min)
SAMPLE NO.
A
B

Figure 25 A

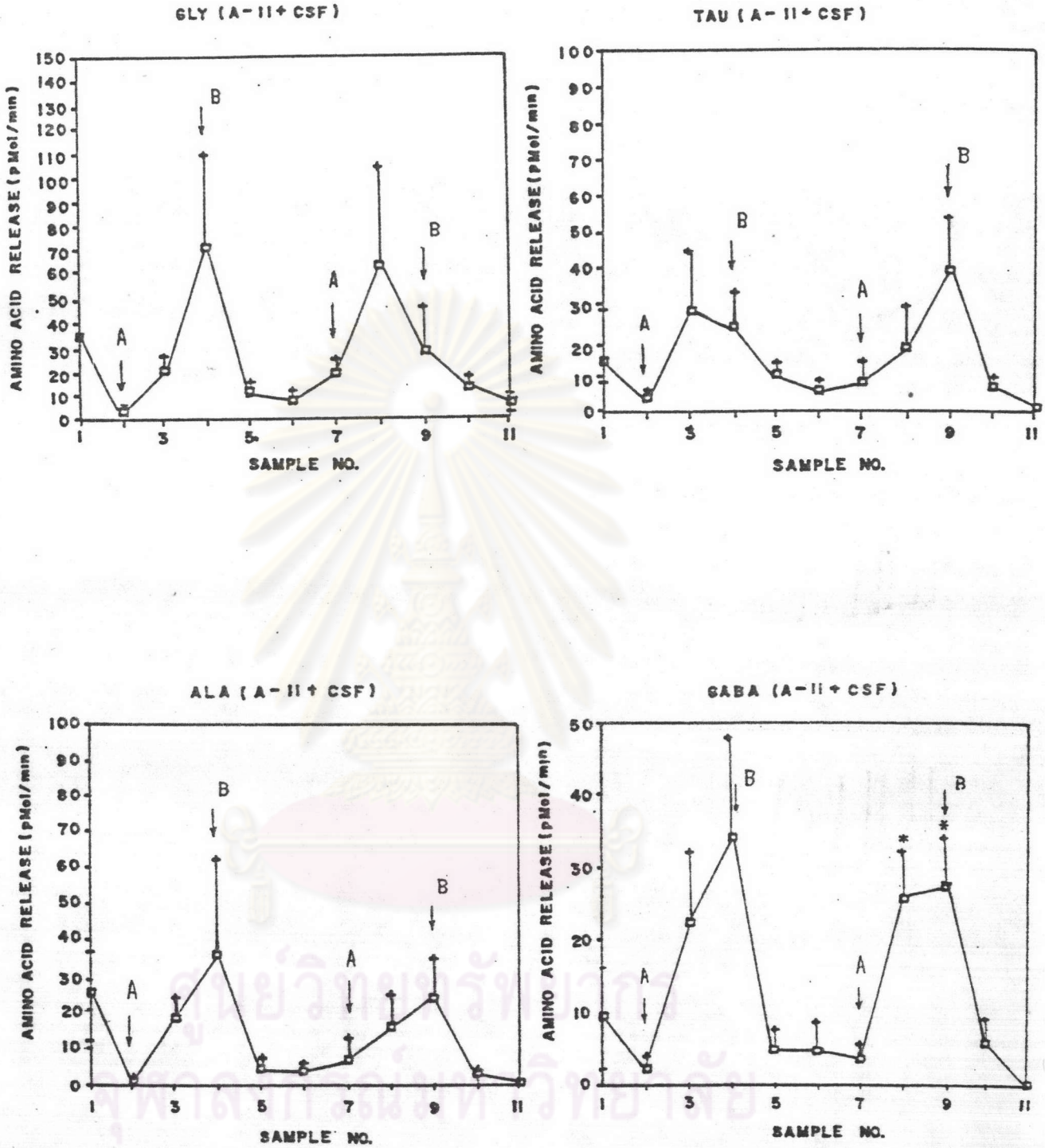
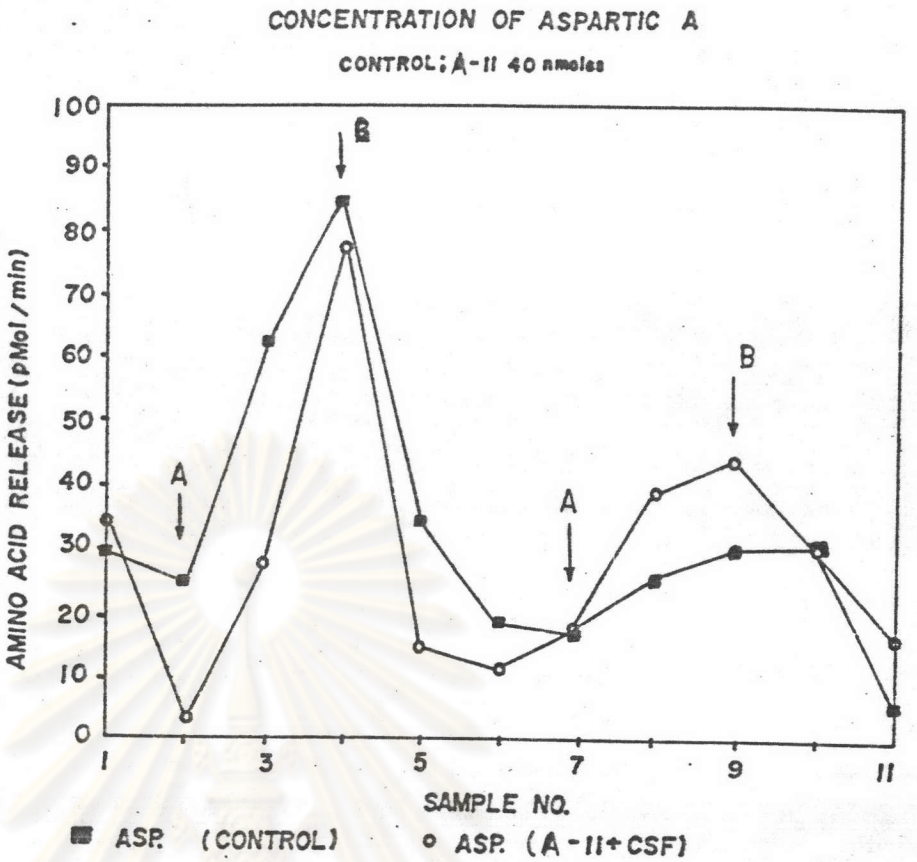

GLY (A-II+CSF)
TAU (A-II+CSF)
ALA (A-II+CSF)
GABA (A-II+CSF)
AMINO ACID RELEASE (pMol/min)
SAMPLE NO.
A
B

Figure 25 B


CONCENTRATION OF ASPARTIC A
CONTROL; A-II 40 nmoles
AMINO ACID RELEASE (pMol / min)
SAMPLE NO.
ASP. (CONTROL)
ASP. (A-II+CSF)

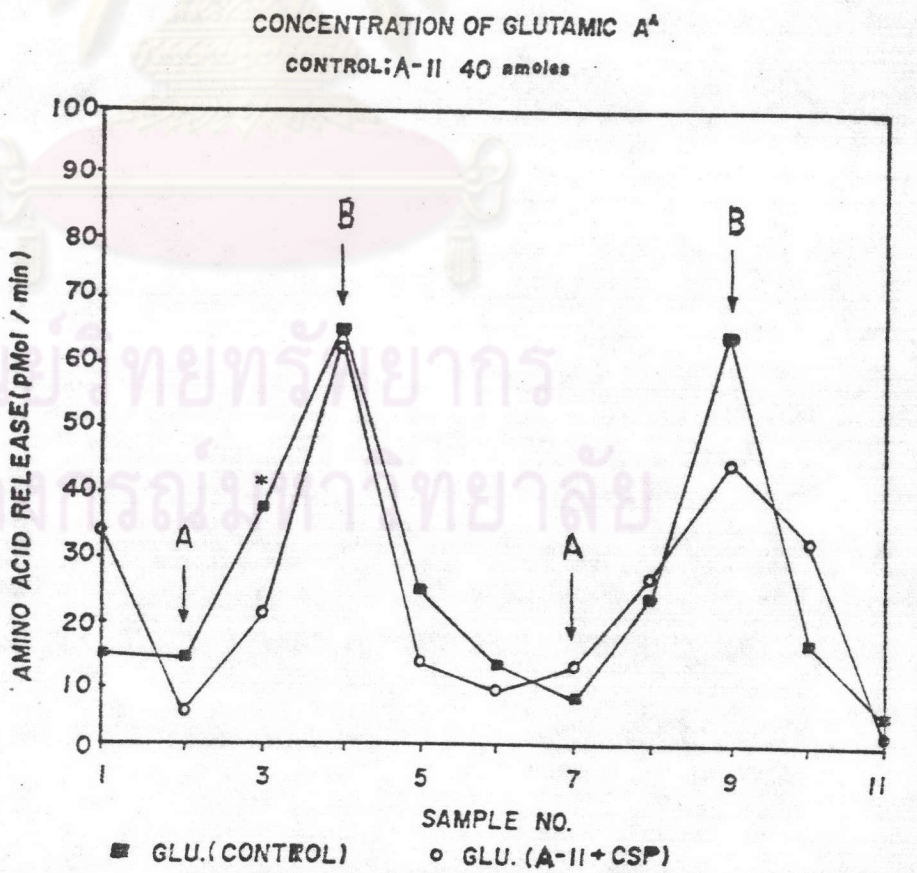
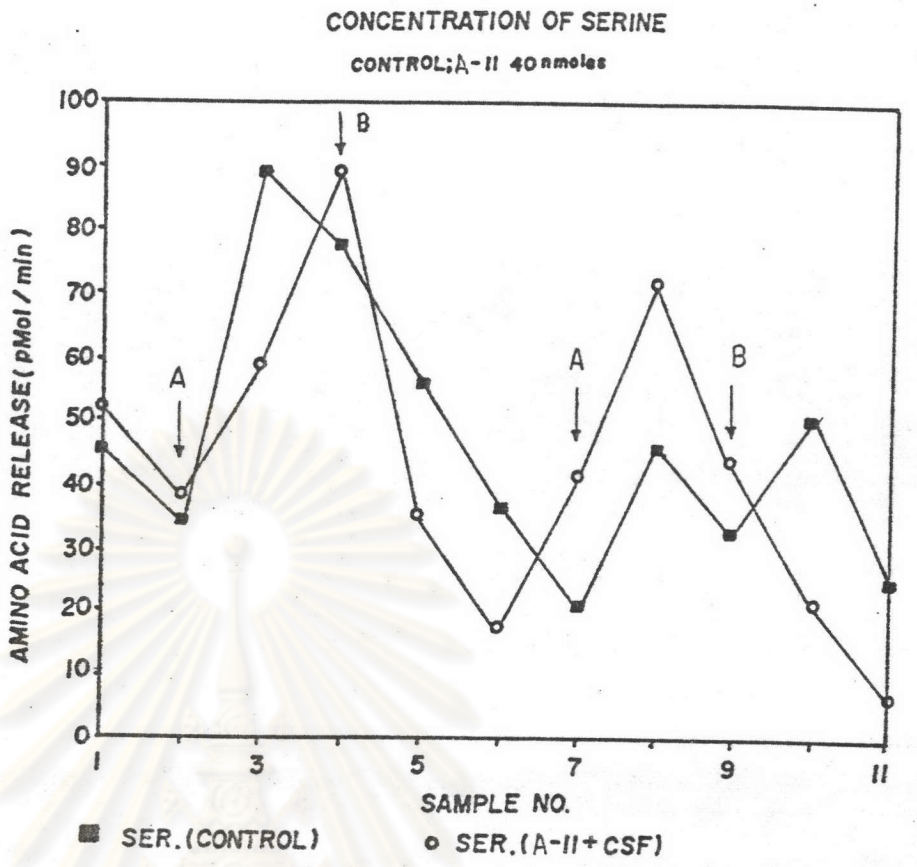

CONCENTRATION OF GLUTAMIC A
CONTROL; A-II 40 nmoles
AMINO ACID RELEASE (pMol / min)
SAMPLE NO.
GLU. (CONTROL)
GLU. (A-II+CSF)

Figure 26 A


CONCENTRATION OF SERINE
CONTROL;A-II 40nmoles
AMINO ACID RELEASE(pMol/min)
SAMPLE NO.
SER.(CONTROL)
SER.(A-II+CSF)

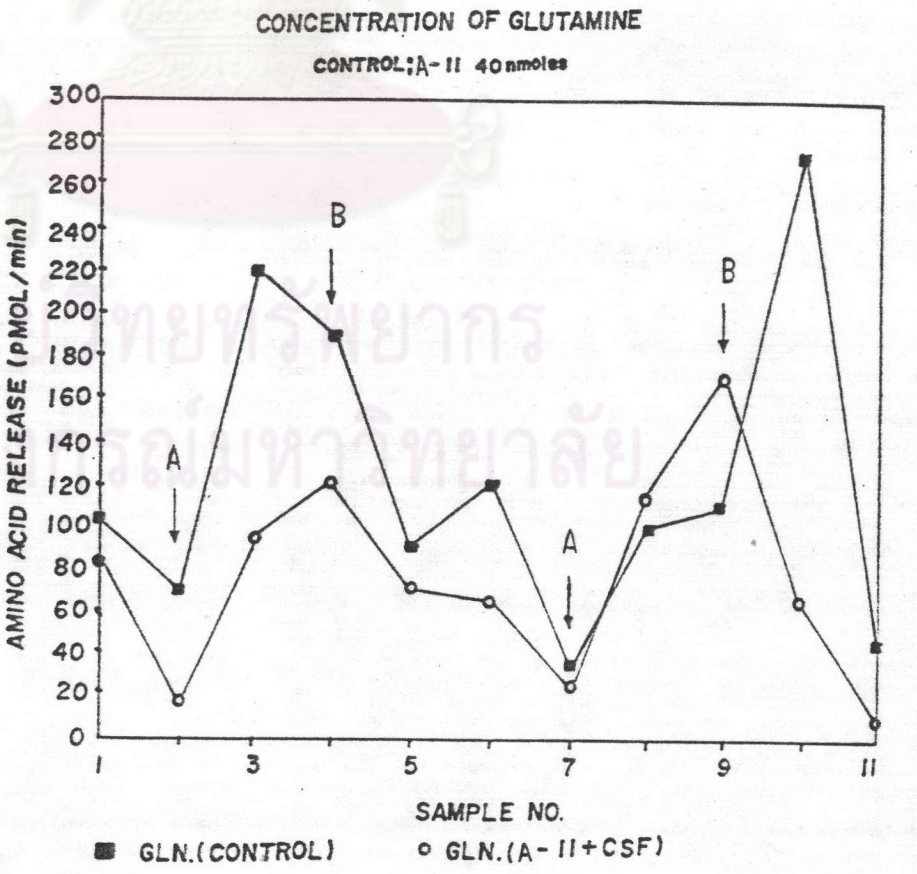
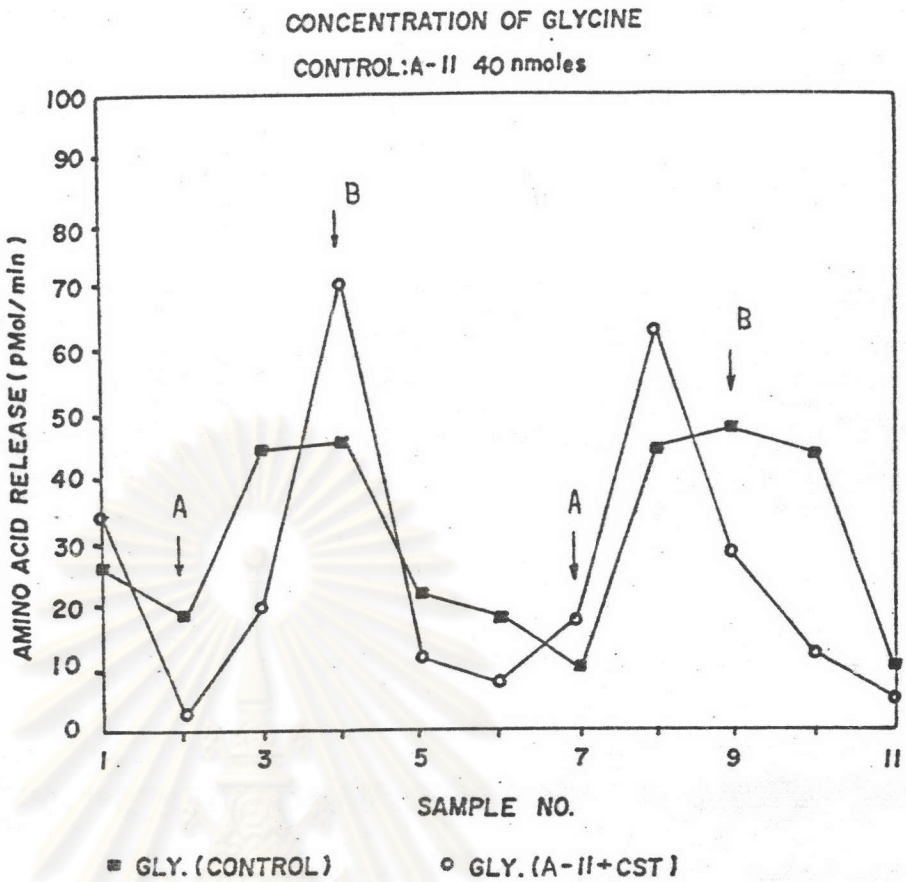

CONCENTRATION OF GLUTAMINE
CONTROL;A-II 40nmoles
AMINO ACID RELEASE (pMOL/min)
SAMPLE NO.
GLN.(CONTROL)
GLN.(A-II+CSF)

Figure 26 B


CONCENTRATION OF GLYCINE
CONTROL:A-II 40 nmoles
AMINO ACID RELEASE (pMol/ min)
100
90
80
70
60
50
40
30
20
10
0
A
B
A
B
1
3
5
7
9
11
SAMPLE NO.
GLY. (CONTROL)
GLY. (A-II+CST)

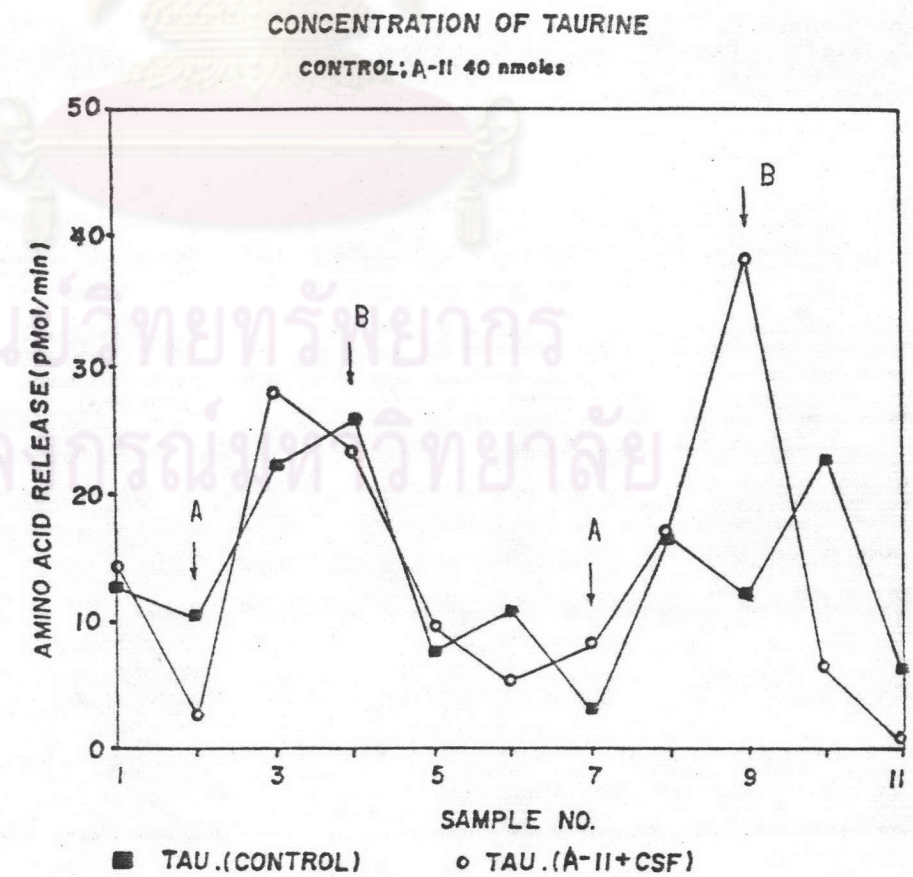
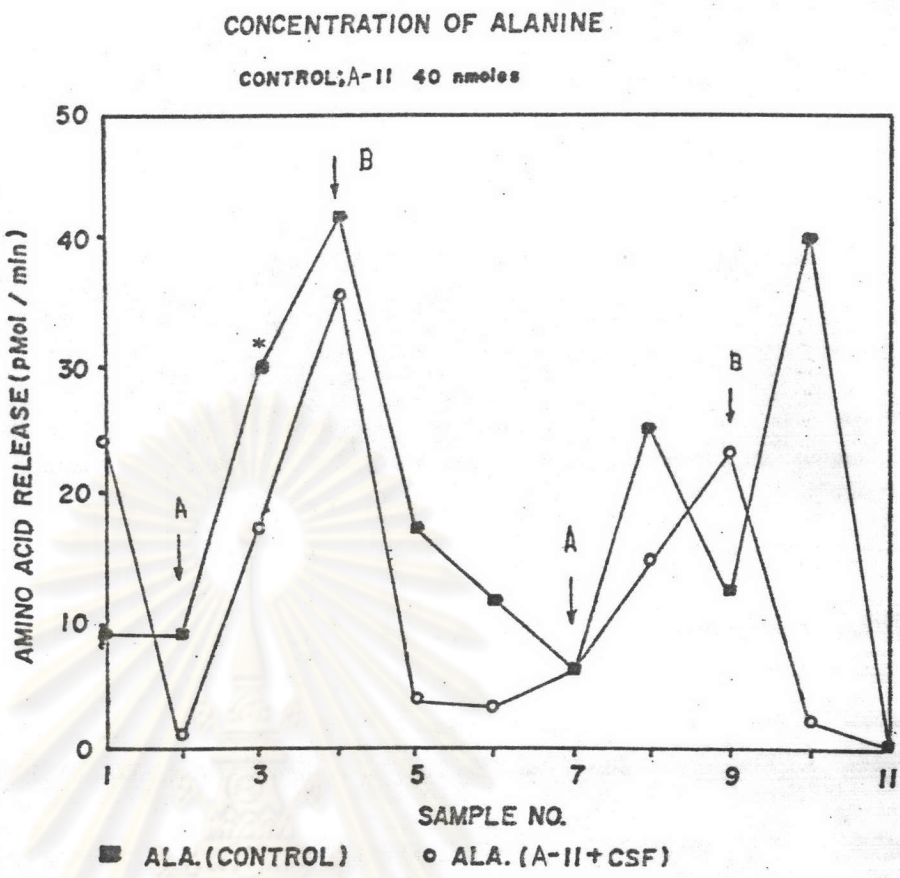

CONCENTRATION OF TAURINE
CONTROL; A-II 40 nmoles
AMINO ACID RELEASE(pMol/min)
50
40
30
20
10
0
A
B
A
B
1
3
5
7
9
11
SAMPLE NO.
TAU.(CONTROL)
TAU.(A-II+CSF)

Figure 26 C


CONCENTRATION OF ALANINE
CONTROL;A-II 40 nmoles
AMINO ACID RELEASE (pMol / min)
SAMPLE NO.
A
B
ALA.(CONTROL)
ALA.(A-II+CSF)

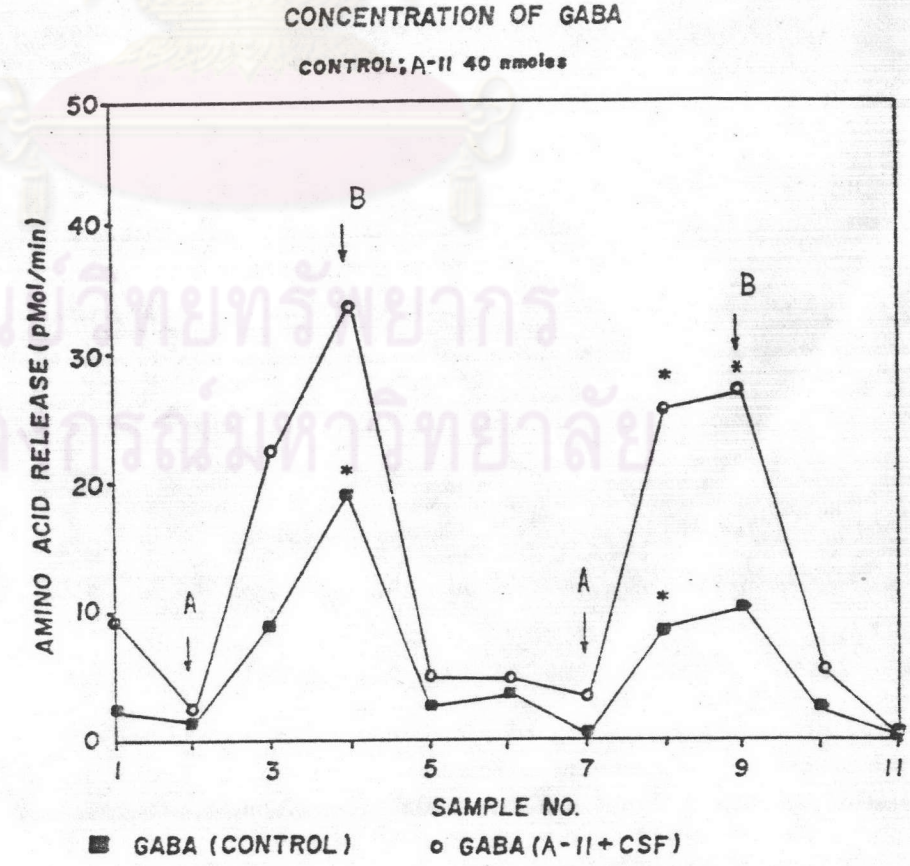

CONCENTRATION OF GABA
CONTROL;A-II 40 nmoles
AMINO ACID RELEASE (pMol/min)
SAMPLE NO.
A
B
GABA (CONTROL)
GABA (A-II+CSF)

Figure 26 D

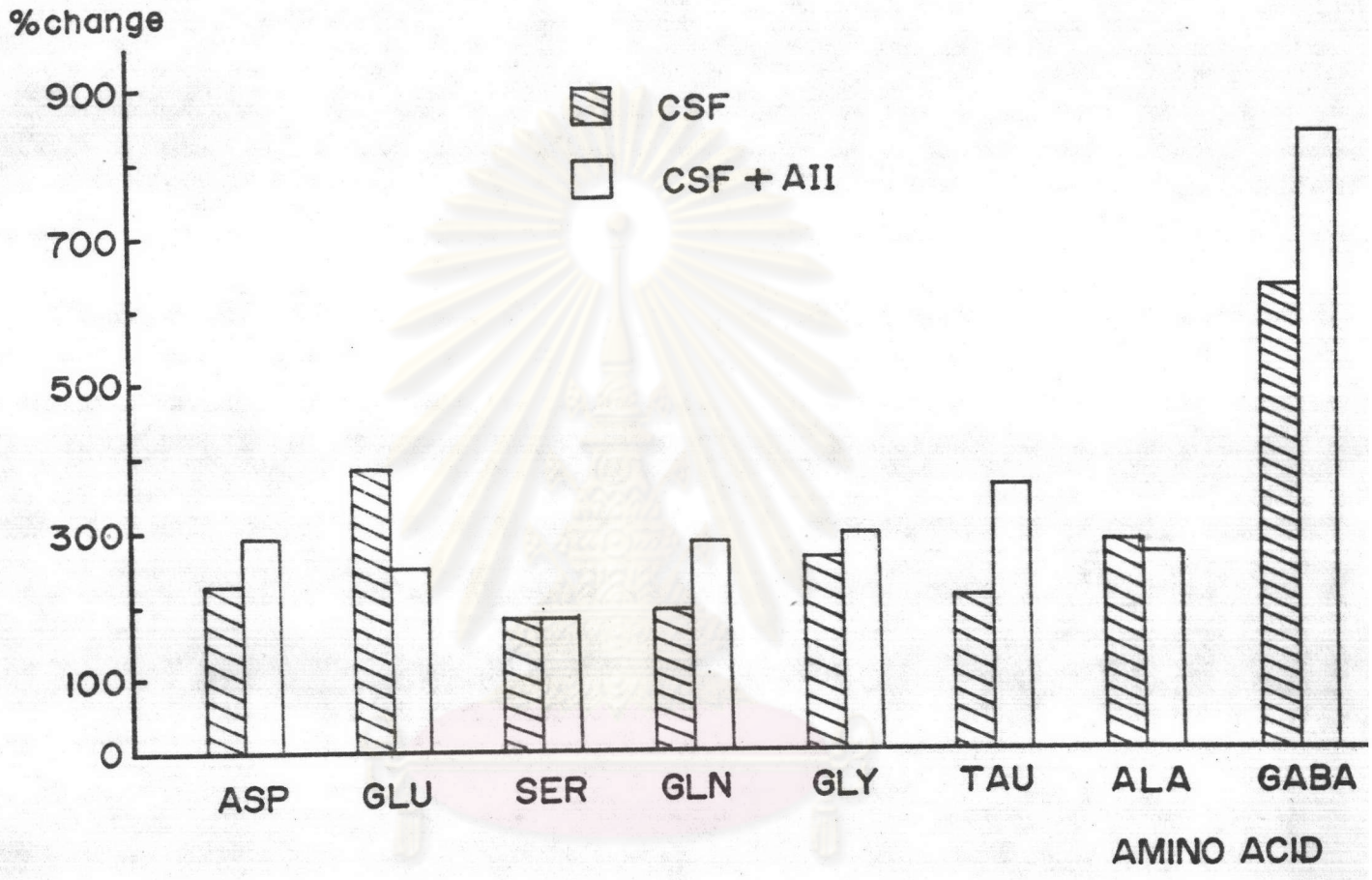


figure 27 % change of amino acid when superfused the rat cerebellar cortex with CSF+AII ☐, compared with CSF superfusion. ▨