CHAPTER IV

RESULTS

1. Amino Acids Analysis

Under the chromatographic conditions described, separation of amino acid contents, both of the perfusate sample and standard solutions were accomplished within approximately 20 minutes. A sample chromatogram of OPA derivatives of a standard solution containing mixture of 8 amino acids (10 nmol/50 µl of each) : aspartic acid (Asp), glutamic acid (Glu), serine (Ser), glutamine (Gln), glycine (Gly), taurine (Tau), alanine (Ala) and γ-aminobutyric acid (GABA) is illustrated in Fig.9. The area of the OPA derivative of each peak of the chromatogram was directly proportional to the amount of the OPA derivative of each standard with linearity reliable within the range 50 pmol to 2500 pmol amino acid content (Fig. 10). The coefficient of variation of the peak area of each amino acid is shown in Table 1.

2. Microdialysis Experiments.

From *in vitro* test, the amino acids differed in their recoveries expressed as percentage value as shown in Table 2. These values were used to calculate their actual concentration in perfusate sample recovered from the vestibular nuclei. Fig. 9 shows the typical pattern of chromatogram of perfusate sample obtained from correct placement of microdialysis probe within

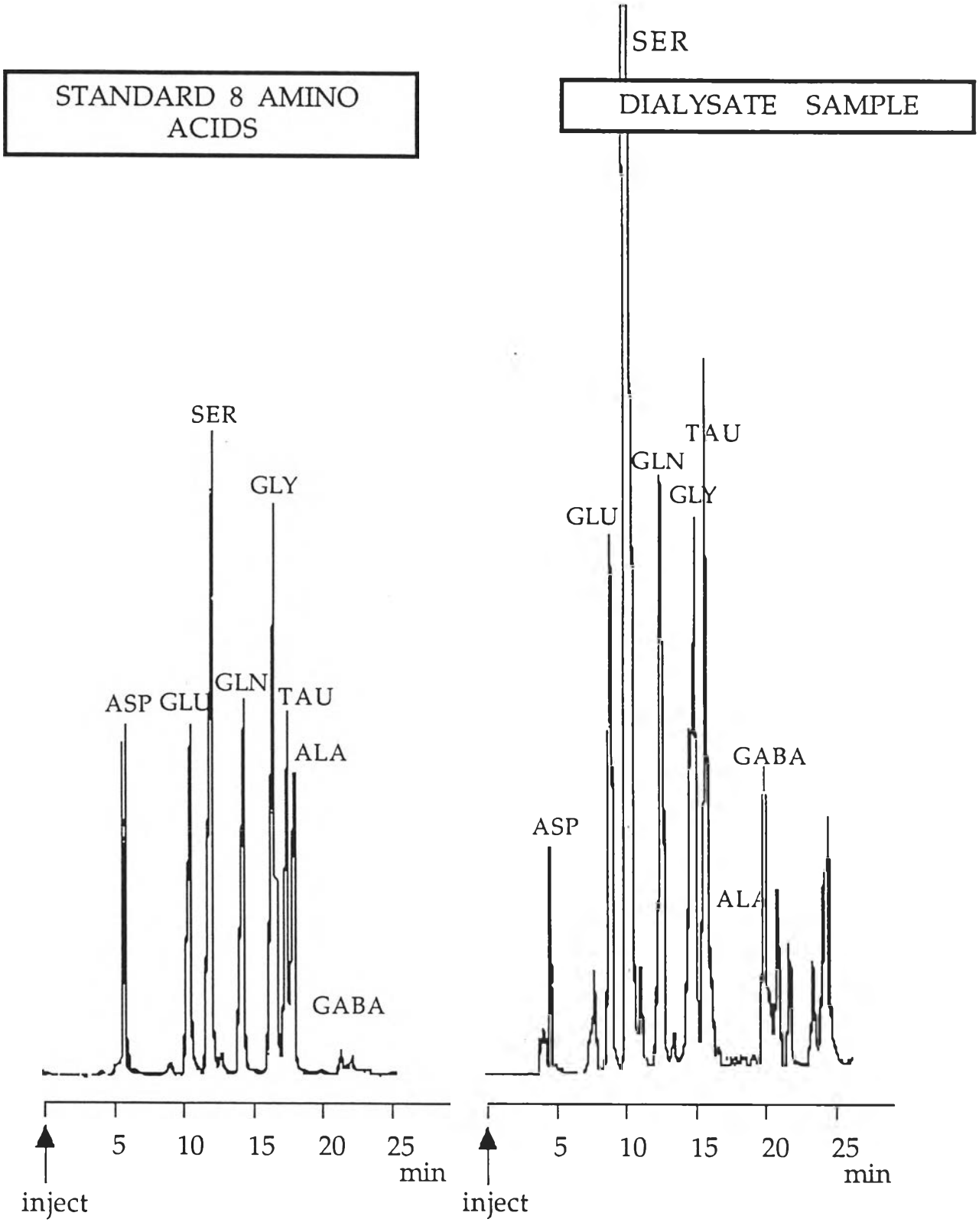
Table.1 Coefficient of variation (C.V.) of the peak area. Concentration of each component is 250 pmol; number of determination =4

Compound	C.V.(%)
Aspartic acid	5.68
Glutamic acid	1.37
Serine	13.53
Glutamine	10.71
Glycine	4.86
Taurine	6.97
Alanine	1.88
GABA	5.21

Table 2. Recovery of each standard amino acid (10 nmol/50µl) at flow rate 2.0 µl/min. Data are average ±S.E.M. (n =10)

Compound	Molecular weight	Recovery (%)
Aspartic acid	133.1	12.93±0.99
Glutamic acid	147.1	20.13±1.00
Serine	105.1	22.35±1.77
Glutamine	146.1	22.77±1.56
Glycine	75.07	20.39±1.22
Taurine	125.1	20.45±1.33
Alanine	89.09	18.56±1.19
GABA	103.1	34.36±2.89

Fig. 9. Measurement of eight amino acids in standard sample and dialysis perfusate from the vestibular nuclei using HPLC with fluorescence detector and microdialysis probe. Chromatogram **A** shows the separation of eight amino acids standard (10 nmol/50 µl) while chromatogram **B** is a dialysis perfusate from a normal vestibular nuclei (30 µl collected after starting perfusion for 30 min). Amino acids were separated by HPLC column using 0.01 M phosphate buffer, pH 7.3 and methanol as mobile phase with a flow rate 0.5 ml/min. and were detected using an excitation wavelength of 330 nm and emission wavelength of 418 nm.


STANDARD 8 AMINO ACIDS
SER
GLY
ASP GLU
GLN
TAU
ALA
GABA
5
10
15
20
25
min
inject
SER
DIALYSATE SAMPLE
TAU
GLN
GLY
GLU
GABA
ASP
ALA
5
10
15
20
25
min
inject

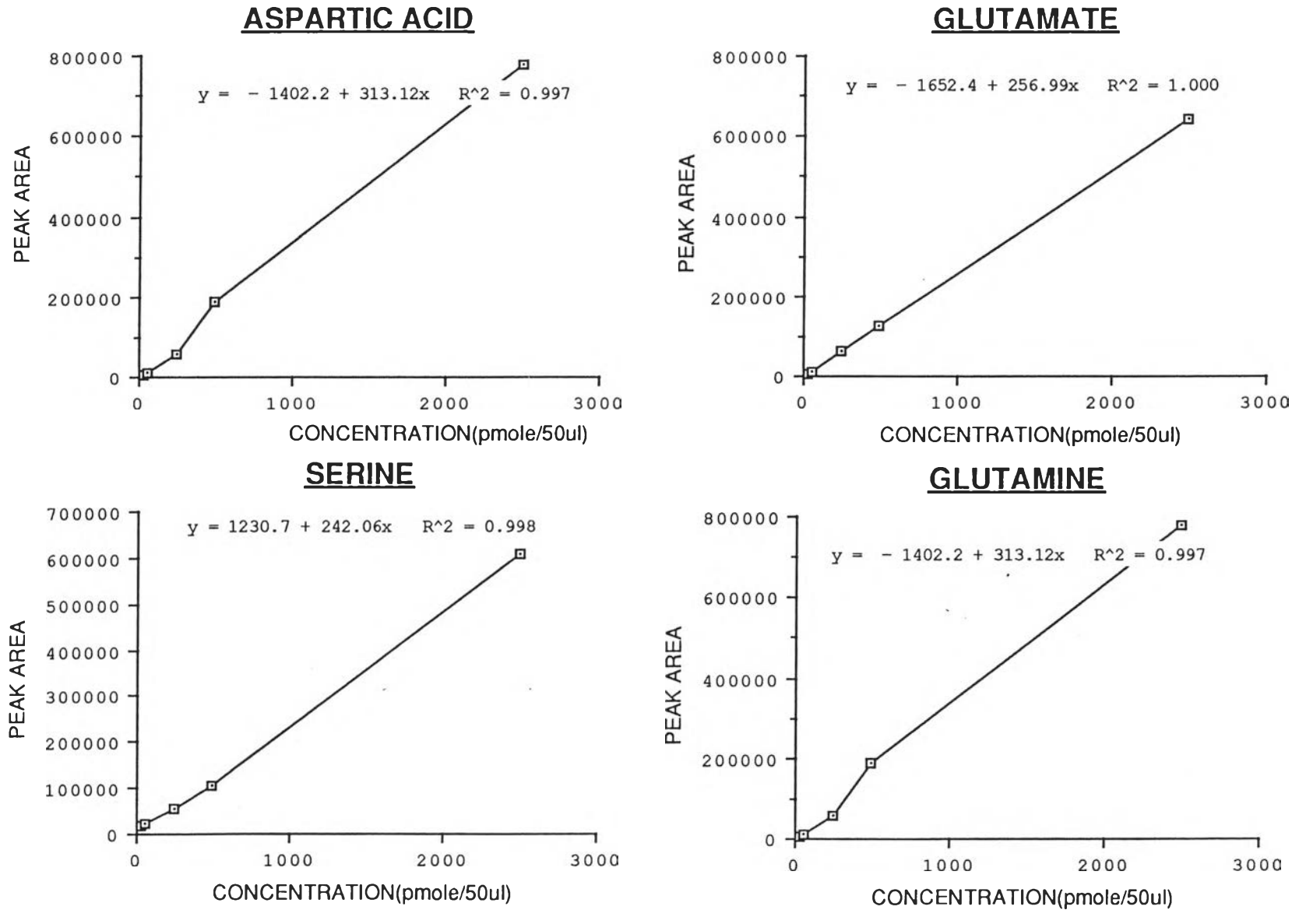
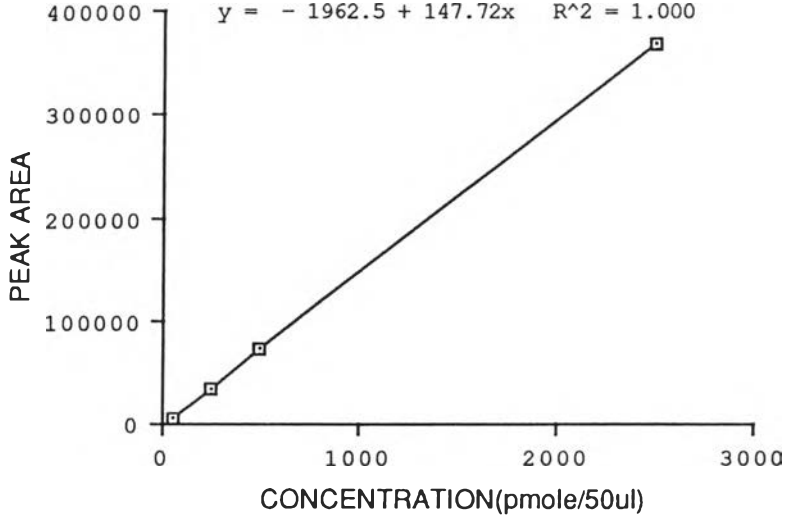
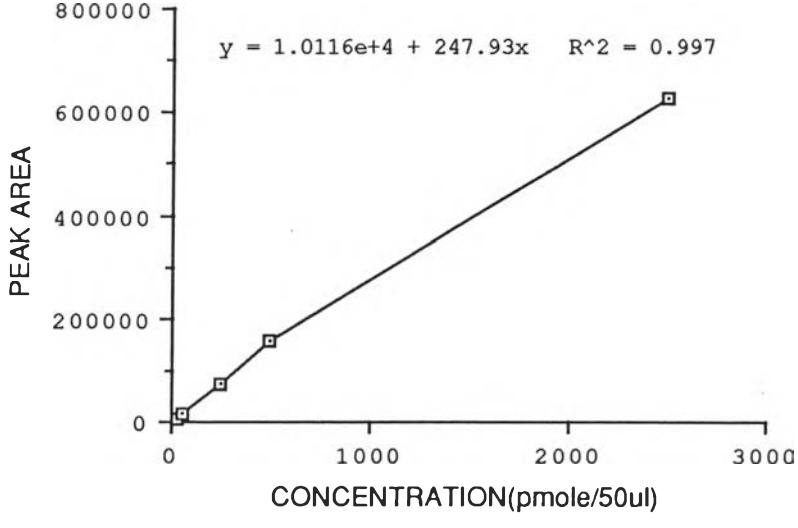


Figure 10. Standard curve of amino acids measurement. Peak area refers to area under each amino acids peak.

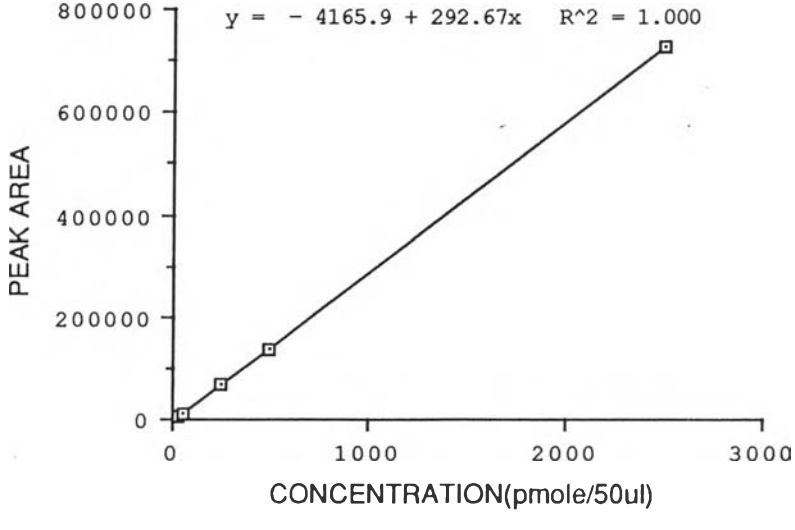
GLYCINE



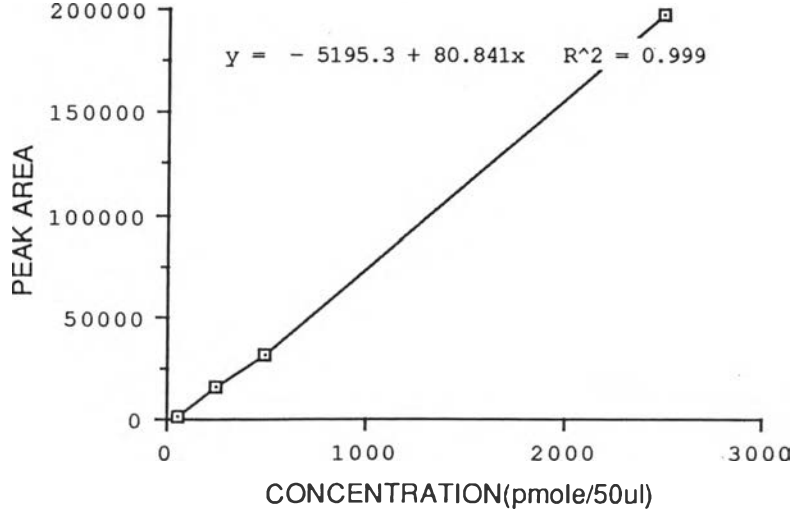
TAURINE



ALANINE



GABA



the limit of the vestibular nuclei, histological section illustrated in Fig.11. By contrast, incorrect placement of the probe which located outside the limit of the vestibular nuclei yielded less amount of amino acids release as shown in Fig. 12.

3. Spontaneous Release of Endogenous Amino Acids.

After the microdialysis probe was implanted into the vestibular nuclei and superfused continuously with aCSF, the first two fractions were discarded to avoid effects of injury induced release. The amount of various endogenous amino acids recovered in the initial perfusate fractions were still slightly higher than those of the subsequent fractions perfused during a period of 120 min, although the difference is insignificant. The pattern of endogenous amino acids release was consistent in all perfusate fraction (Fig.13 and Table 3).

4. Effect of Electrical Stimulation on Amino Acids Release.

These experiments were carried out in order to induce release of the endogenous amino acids from vestibular nerve terminal by electrical stimulation (n=5). By electrophysiological investigation, the field potentials (Fig.14) evoked in the vestibular nuclei of the rat following stimulation of the ipsilateral vestibular nerve resemble those previously described by Precht and Shimazu, 1965; Sangchantra,1986 and Warunee, 1987. The stimulation was carried out after perfusing with aCSF for 30 min using frequency 50 Hz, current 1 mA for 5 min. The pattern of release of endogenous amino acids of control fraction and those after nerve

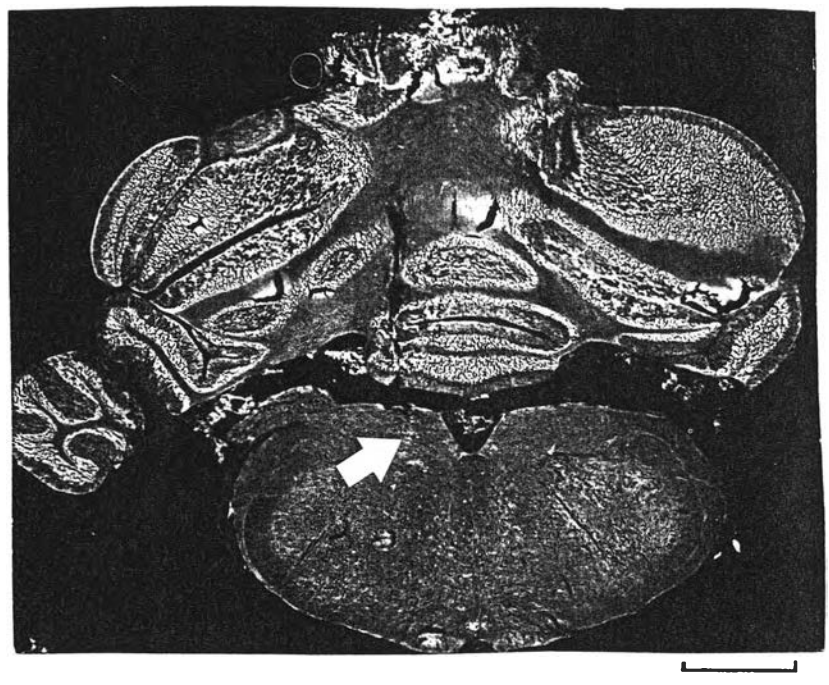


Figure 11. Coronal section of the rat brain which the microdialysis probe tip located inside the vestibular nuclei, at the arrow.

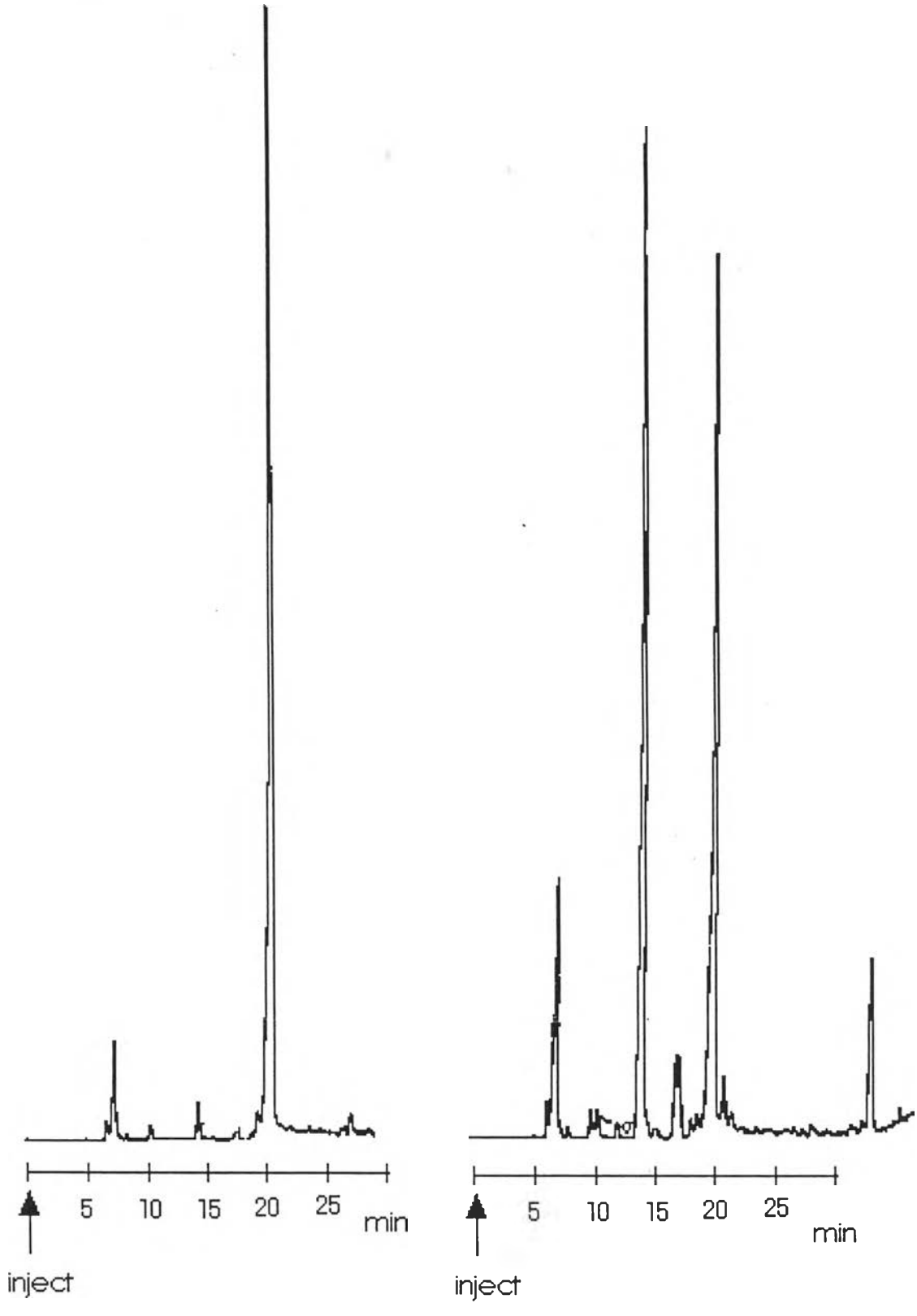
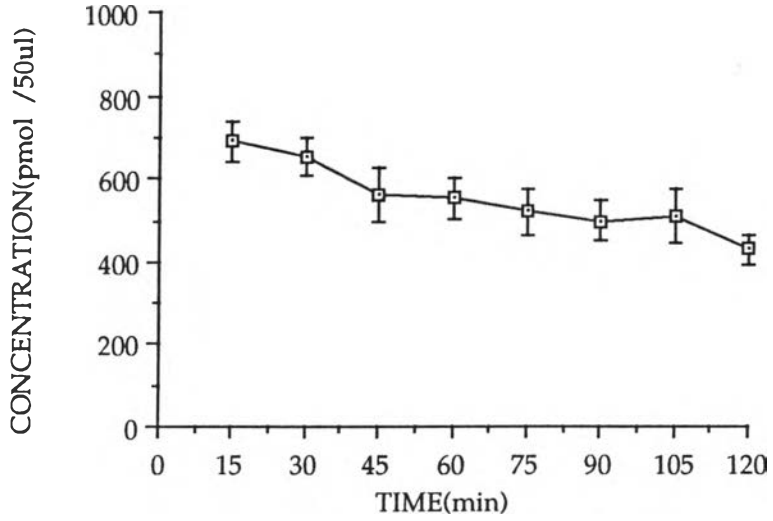


Figure 12. Chromatograms of perfusate samples from incorrect placement of the microdialysis probe.

Figure 13. Time course of spontaneous release of Asp, Glu, Ser, Gln, Gly, Tau, Ala and GABA recovered in the vestibular nuclei of the normal rats by microdialysis probe. Each point represents the mean release of amino acid in pmol/50μl during 15 min collection in 8 experiments and the S.E.M. is shown by the vertical bar.

ASPARTATE



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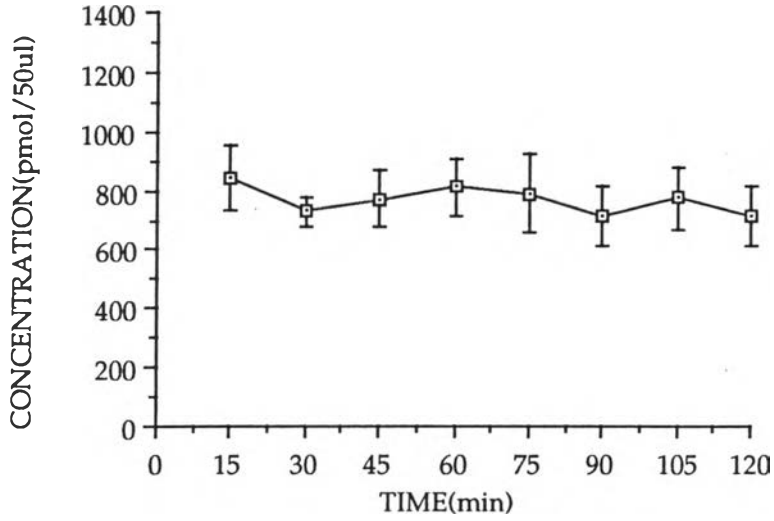
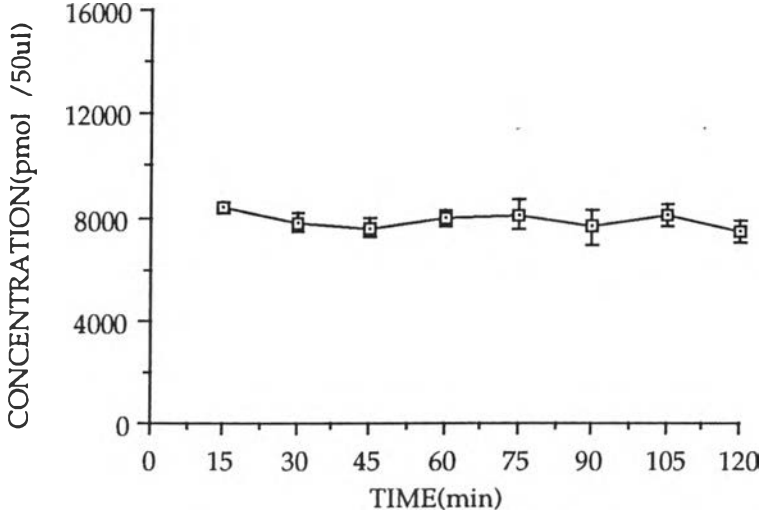
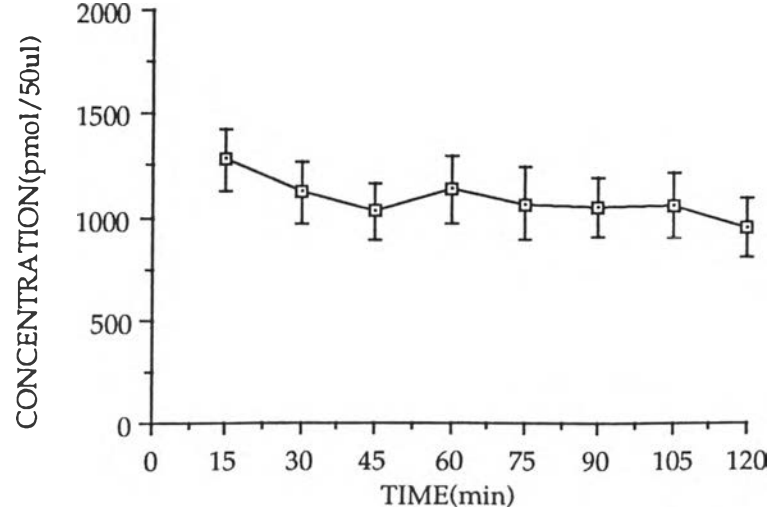


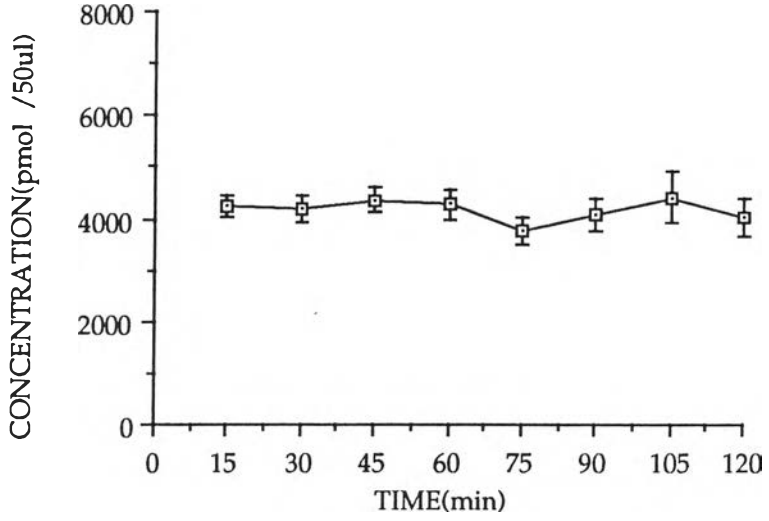
SERINE



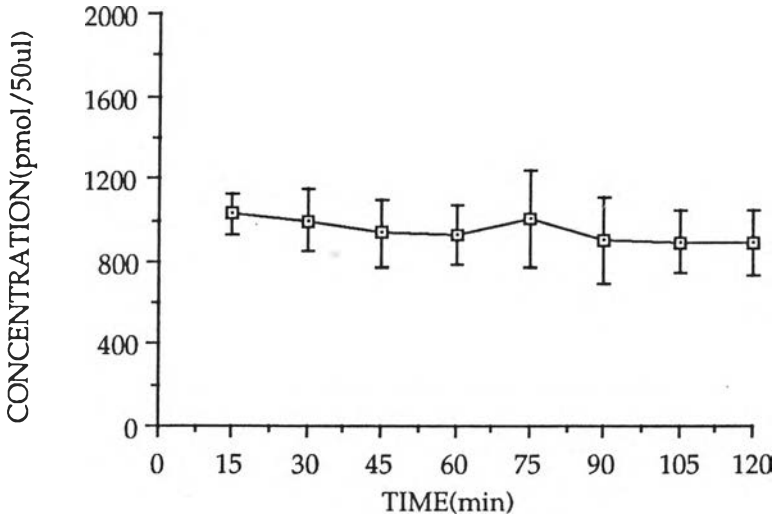
GLUTAMINE



GLYCINE



TAURINE



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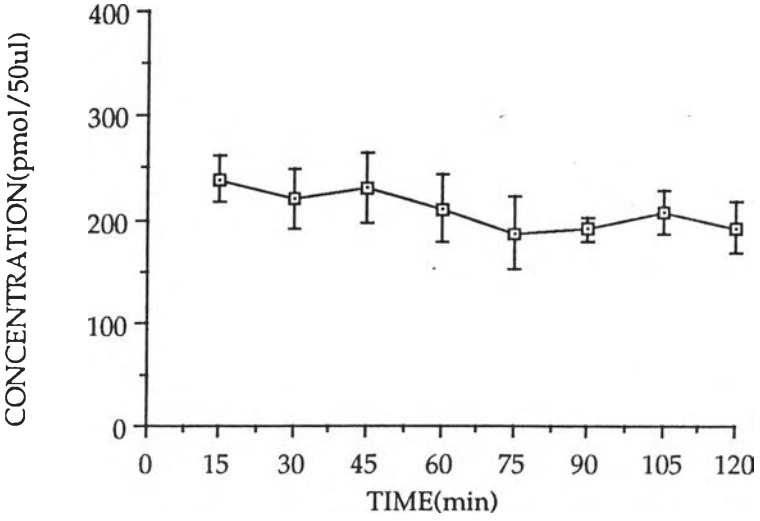
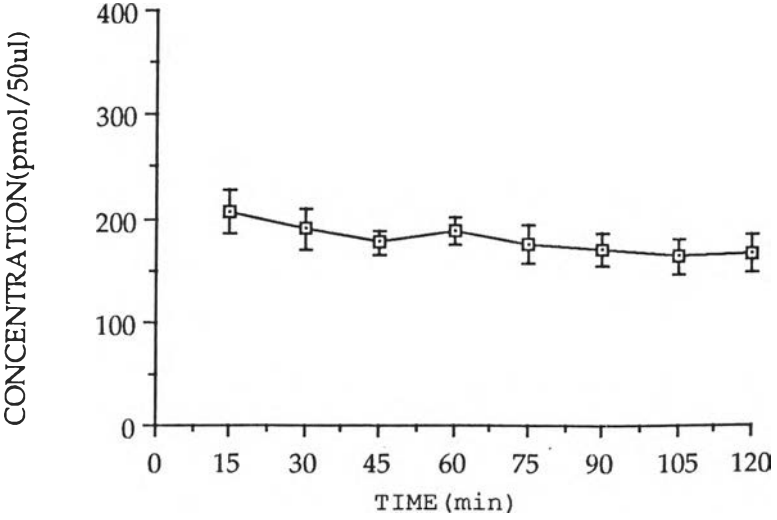


GABA



TIME(min)	15	30	45	60	75	90	105	120
COMP.	aCSF							
ASP	691.87 ±48	655.25 ±45	562.22 ±65	552.41 ±46	521.13 ±54	496.95 ±49	510.12 ±63	428.82 ±36
GLU	842.40 ±109	728.93 ±49	770.64 ±97	810.16 ±97	788.95 ±131	715.10 ±100	775.16 ±106	711.55 ±99
SER	8352.36±216	7778.86±339	7535.65±369	7952.24±349	8092.86±558	7587.63±701	8024.63±396	7443.02±445
GLN	1274.74±147	1118.24±155	1029.62±137	1132.58±160	1063.12±174	1049.10±142	1061.53±160	954.88±146
GLY	4231.00±212	4186.01±286	4344.43±232	4271.94±287	3770.35±270	4070.85±296	4415.00±492	4042.15±366
TAU	1028.64 ±98	997.78 ±148	936.45 ±160	927.46 ±144	1009.53 ±238	905.03 ±209	894.25 ±155	886.66 ±159
ALA	238.85 ±21	219.40 ±28	229.35 ±33	210.40 ±31	186.72 ±36	189.91 ±12	207.25 ±20	191.63 ±24
GABA	206.43 ±21	189.86 ±20	176.91 ±12	188.30 ±13	176.14 ±18	169.95 ±16	164.33 ±16	167.25 ±17

Table 3. Time course of spontaneous release of endogenous amino acids in vestibular nuclei of the normal rats. Data are expressed as mean (pmol/50μl)±S.E.M.

stimulation are shown in Fig.14. In the successful experiment with correct placement of the stimulating electrode, electrical stimulation produced a significant increase of release of aspartate and glutamate at $p<0.05$ comparing with spontaneous release while no significant increase in the efflux of the others was observed (Fig.15 and Table 4). The enhanced release occurred during 3-4 collected fractions when the nerve was stimulated and decreased to the basal level as the initial fractions in the 6th fraction.

5. Effect of Nerve Lesion on Amino Acids Release.

Unilateral vestibular nerve lesion was done by electrolytic lesion as previously described. The rats were divided into three groups according to different survival times before running microdialysis experiment. All of the vestibular nerve lesion were performed on the left side. In acute lesion group, at least 2 fractions of control dialysate were collected prior to application of electrolytic lesion. Immediately after lesioning, the amount of all endogenous amino acids release in the initial perfusate were slightly higher than those of the control (Fig.17) and gradually decreased in aspartate, glutamate, glycine and taurine content during the time period studied (Fig.18 and Table 5).

In 3 days post lesion group, the rats showed abnormal postural equilibrium and ataxic movement. The samples were collected in the lesioned vestibular nuclei following with the contralateral vestibular nuclei by using the same probe. Figure 18 shows the pattern of release of endogenous amino acids from

Figure 14. Chromatogram obtained from the analysis of perfusate fractions collected *in vivo* in the vestibular nuclei by a microdialysis technique. Those peaks corresponding to the OPA-derivatives of amino acids, identified by comparison of their retention times with those of standard components, have been indicated in the Fig. 9. Chromatogram 'CONTROL' represents the release pattern in the 2nd perfusate fraction obtained during a resting situation, whereas chromatogram 'NERVE STIMULATION' represents the release pattern in the 5th perfusate fraction obtained after electrical stimulation of the vestibular nerve at 50 Hz, 1mA for 5 min.

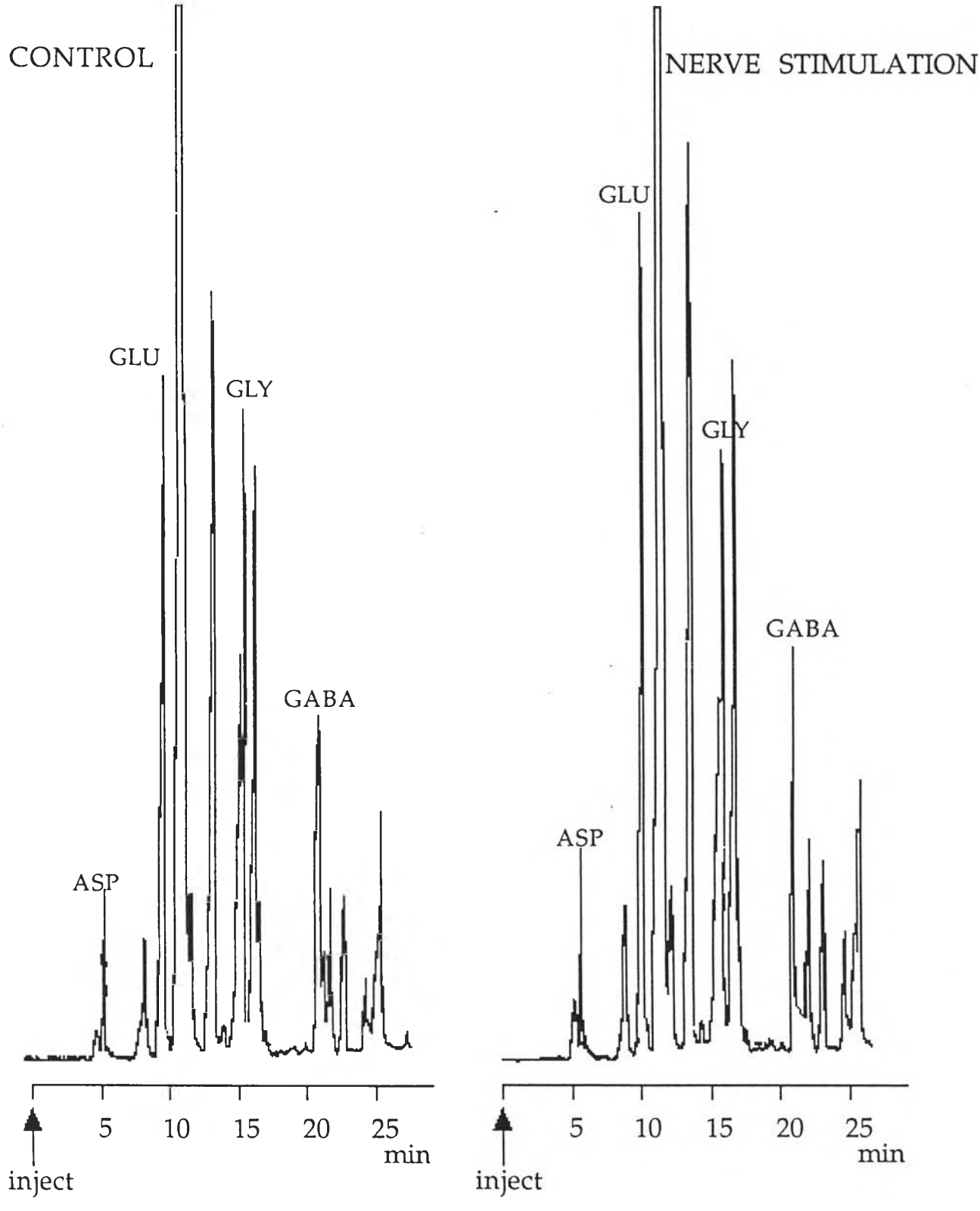
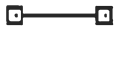

CONTROL
GLU
GLY
GABA
ASP
5
10
15
20
25
min
inject
NERVE STIMULATION
GLU
GLY
GABA
ASP
5
10
15
20
25
min
inject

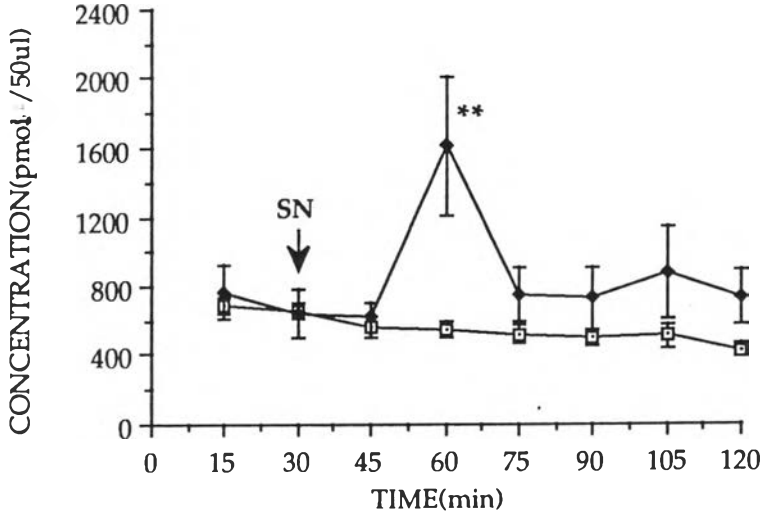
Figure 15. Effects of electrical stimulation of the vestibular nerve on the release of endogeneous amino acids(n=5) compared with spontaneous release in the vestibular nuclei . The stimulation was carried out after perfusing with aCSF for 30 min (2 periods) using 50 Hz, 1mA for 5 min. The figure shows each amino acids such as Asp, Glu, Ser, Gln, Gly, Tau, Ala and GABA recovered by microdialysis probe in normal rats during 15 min fractions. Each point represents the mean release of amino acid (pmol/50µl) and the S.E.M. is shown by the vertical bar. n = number of observations. * $p<0.05$, Student's paired t-test. ** $p<0.05$, Student's unpaired t-test.

represent data for each amino acid in the vestibular nuclei of stimulated rats.



represent data for each amino acid in the vestibular nuclei of normal rats.

ASPARTATE



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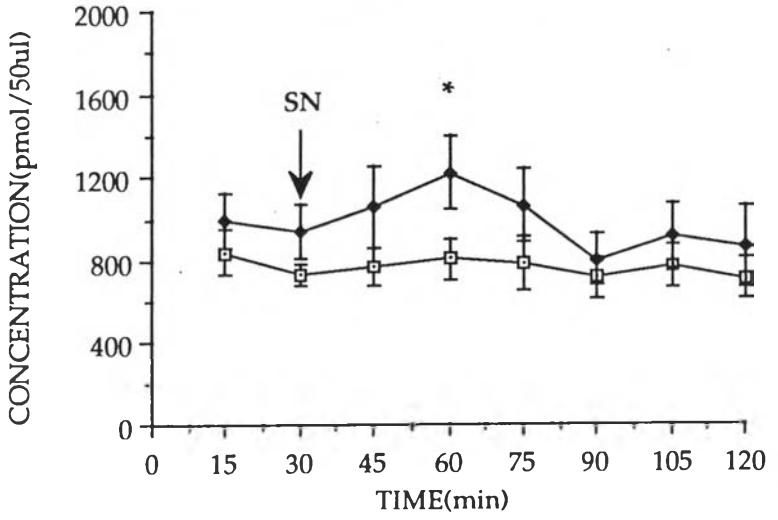

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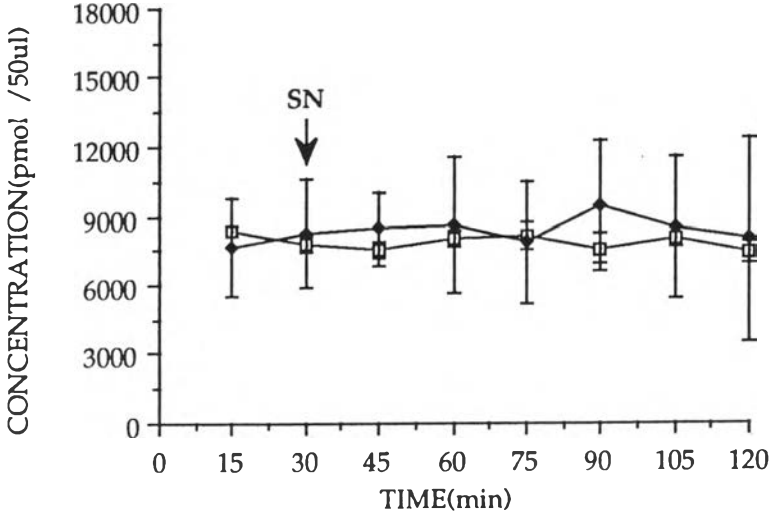

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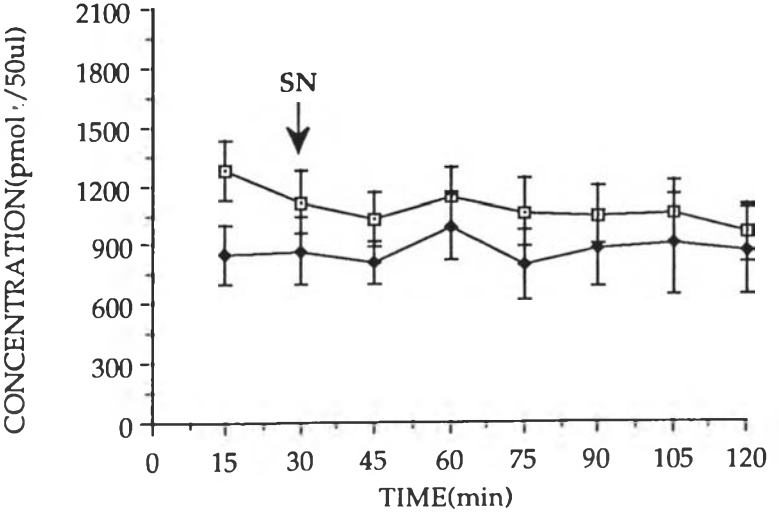

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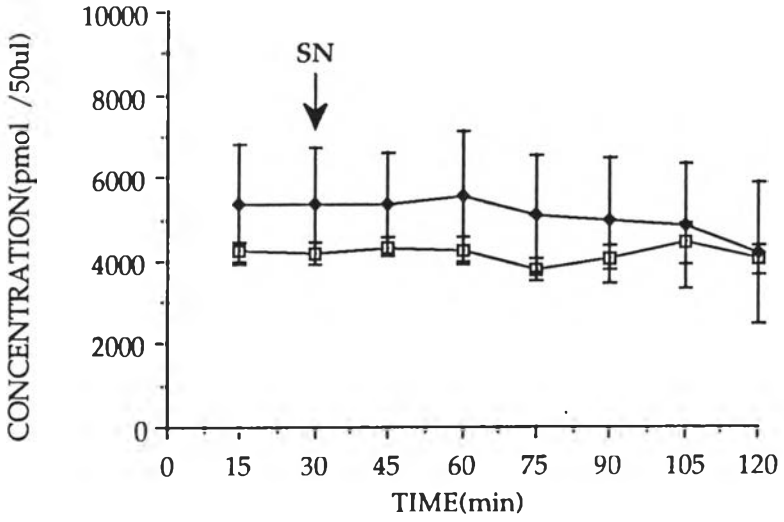

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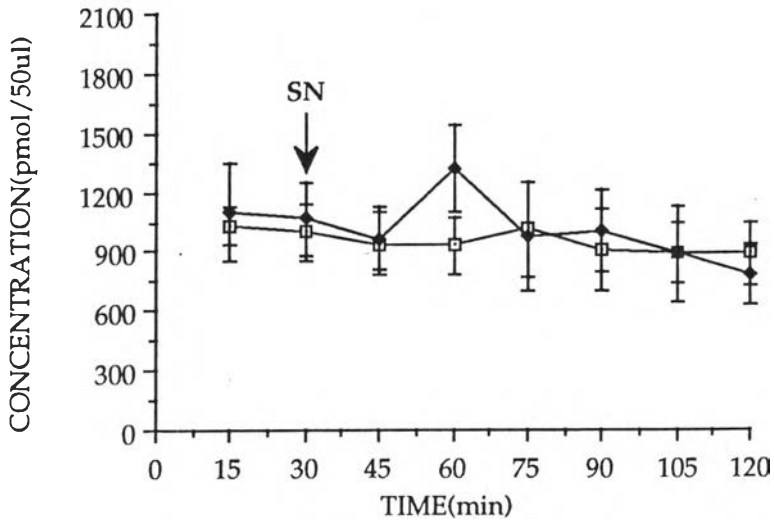

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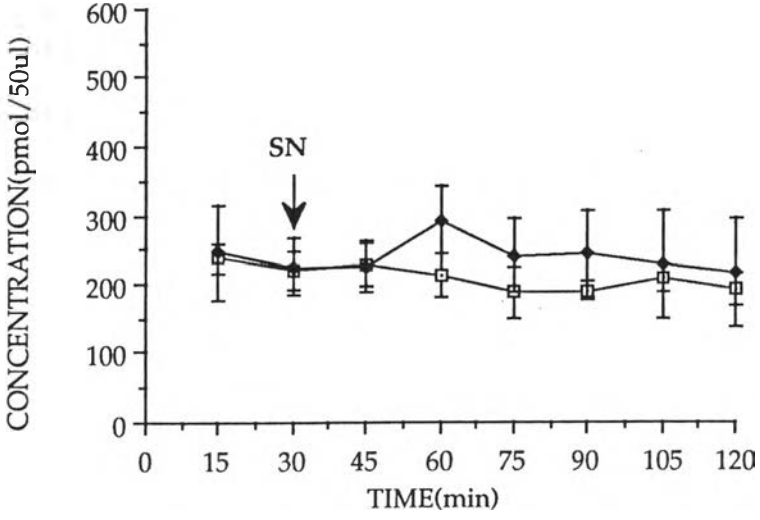
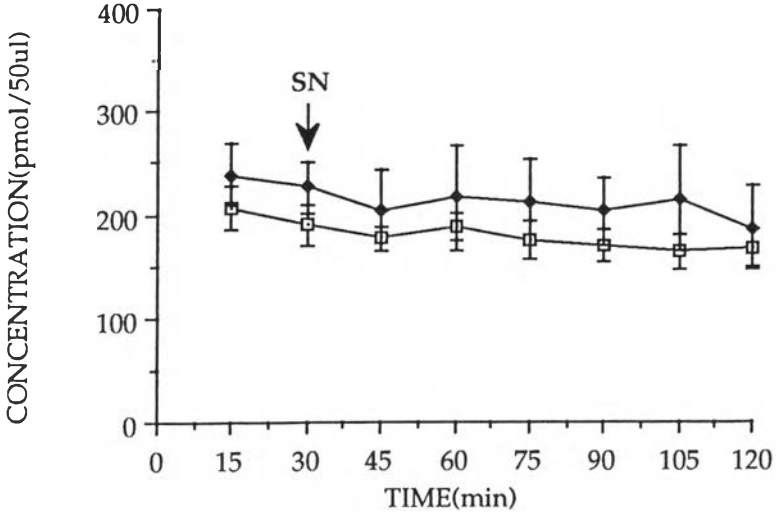


GABA



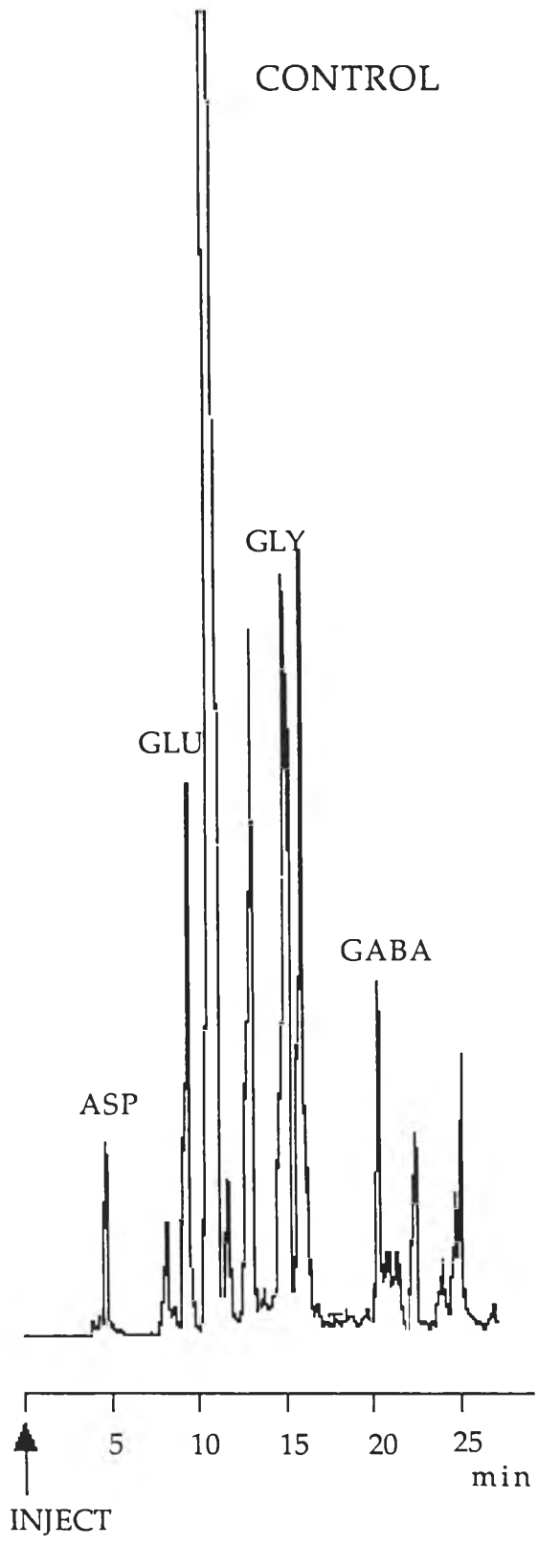
TIME(min)	15	30	45	60	75	90	105	120
COMP.	CONTROL		N.VIII-STIM.		AFTER NERVE STIMULATION			
ASP	769.60 ±150	641.80 ±139	623.60 ±89	1608.2±399**	757.20 ±158	731.20 ±182	881.20 ±268	735.87 ±152
GLU	995.16 ±127	937.59 ±131	1054.93 ±196	1219.6±175*	1063.60 ±175	802.44 ±128	917.02 ±158	865.21 ±194
SER	7665.1±2106	8203.7±2333	8430.4±1569	8568.73 2976	7842.9±2663	9385.4±2824	8467.6±3088	7945.4±4362
GLN	850.38 ±152	867.18 ±171	812.05 ±111	988.06 ±159	797.11 ±172	875.1±192	899.73 ±248	865.29 ±223
GLY	5362.3±1408	5339.4±1385	5352.4±1230	5540.2±1615	5105.1±1402	4954.8±1500	4819.5±1513	4195.4±1686
TAU	1098.2±245	1063.8±190	966.8±162	1319.4±218	970.7±272	1005.3±208	888.07 ±243	779.88 ±155
ALA	245.85 ±67	224.49 ±40	223.88 ±34	291.24 ±49	241.03 ±54	242.68 ±61	226.00 ±78	2115.03 ±79
GABA	238.79 ±29	226.45 ±24	205.05 ±39	215.75 ±50	211.07 ±41	202.69 ±33	215.61 ±50	186.79 ±40

Table 4. Effects of electrical stimulation of vestibular nerve on release of endogenous amino acids. Data are expressed as mean (pmol/50µl) ±S.E.M.

* Significantly different from control at $p<0.05$, Student's paired t-test.

**Significantly different from spontaneous release at $p<0.05$, Student's unpaired t-test.

Figure 16. Chromatogram obtained from the analysis of perfusate fractions collected *in vivo* in the vestibular nuclei of the acute lesion rats. All vestibular nerve lesions were performed on the left side after 2 control fractions had been collected. Chromatogram 'CONTROL' represents the release pattern in the 2nd perfusate fraction obtained from the left vestibular nuclei as control, whereas chromatogram 'ACUTE LESION' represents pattern in the 3rd perfusate fraction obtained from the same side following electrical lesion of vestibular nerve with D.C. current 1mA for 1 min.


CONTROL
GLY
GLU
GABA
ASP
5
10
15
20
25
min
INJECT

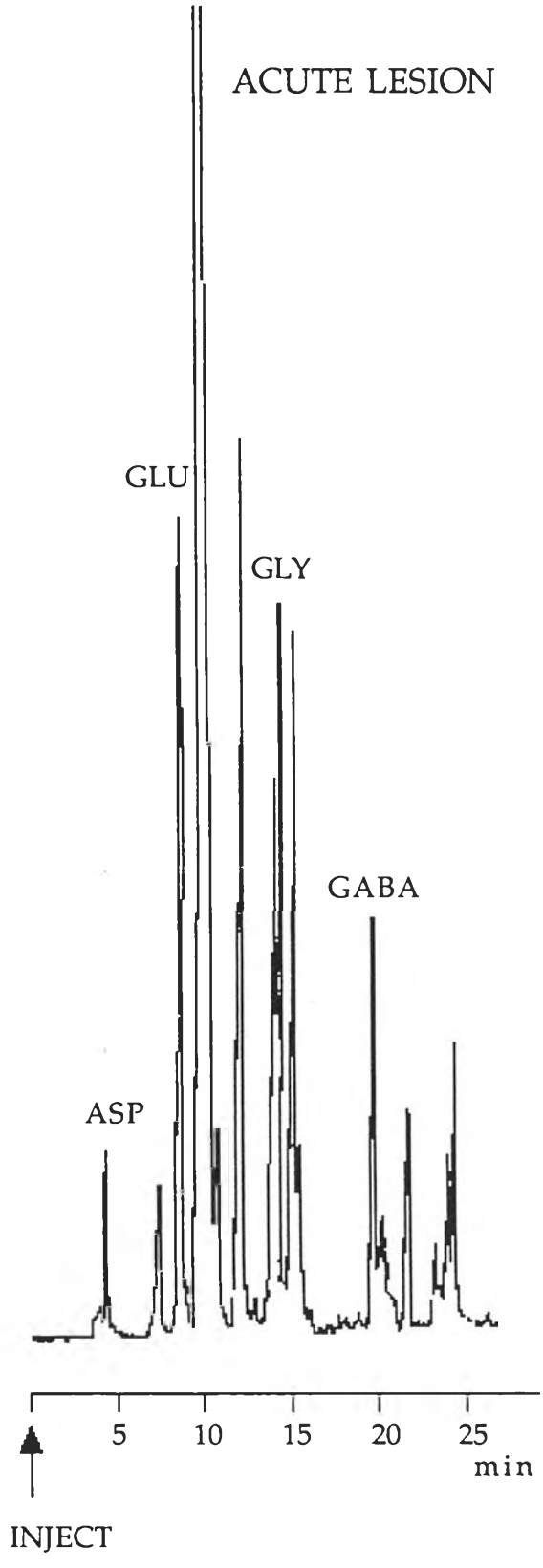
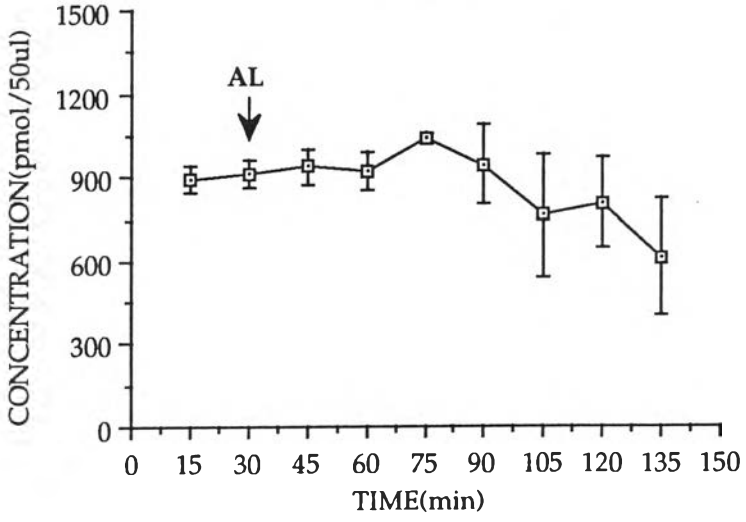

ACUTE LESION
GLU
GLY
GABA
ASP
5
10
15
20
25
min
INJECT

Figure 17. Level of each amino acid in the left vestibular nuclei of acute lesion in normal rats. All vestibular nerve lesion were performed on the left side after two control fractions (n=5). Amino acids examined were Asp, Glu, Ser, Gln, Gly, Tau, Ala and GABA. Values are expressed as mean (pmol/50µl) and the S.E.M. is shown by the vertical bar. n = number of observations.

ASPARTATE



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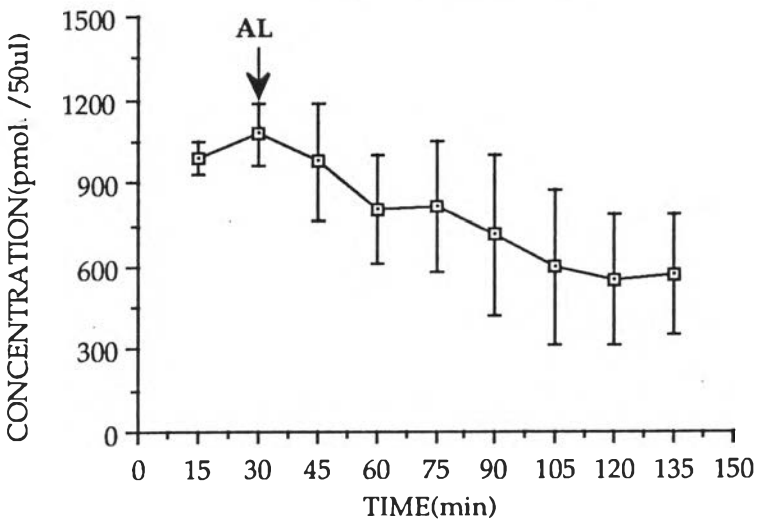

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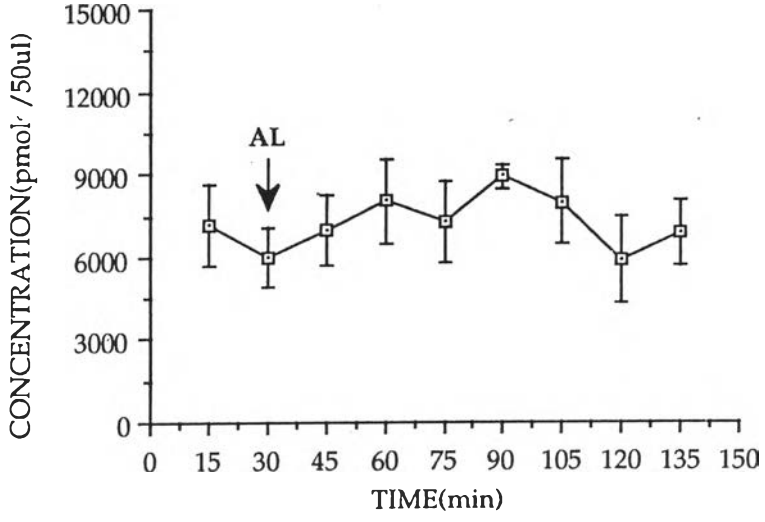
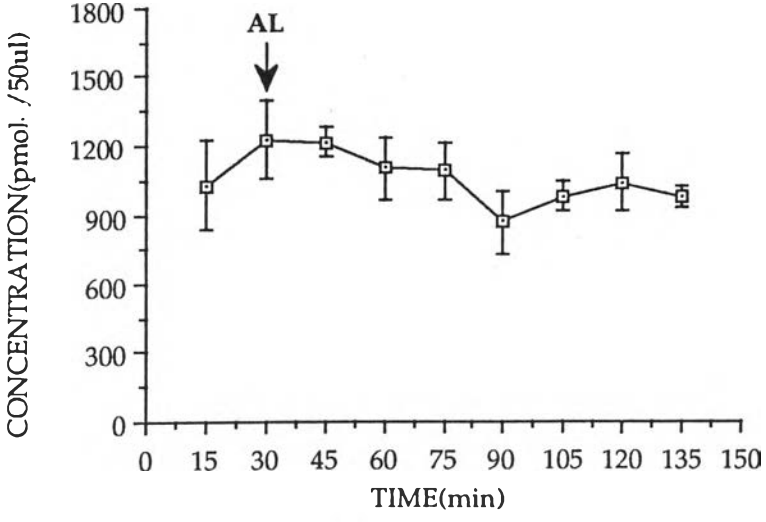
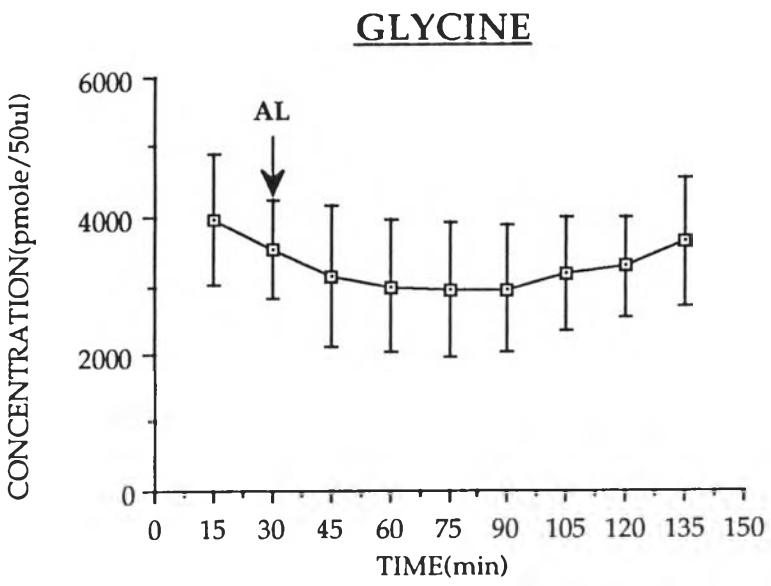
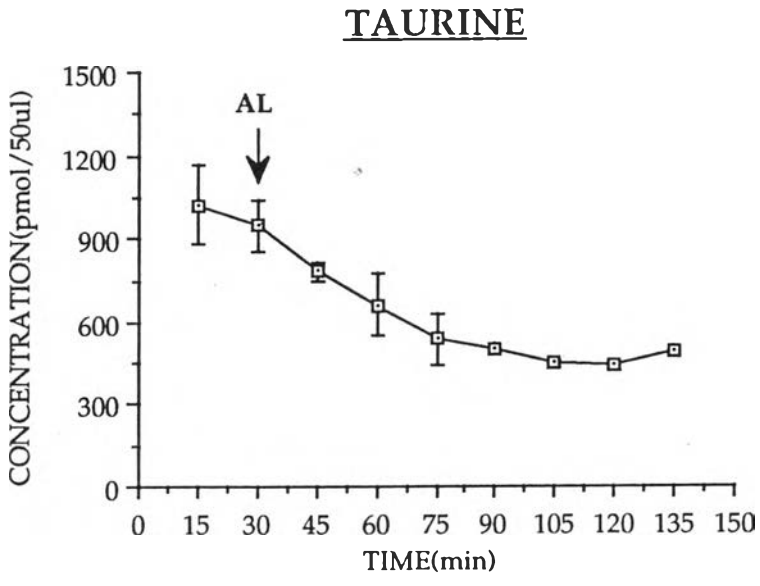
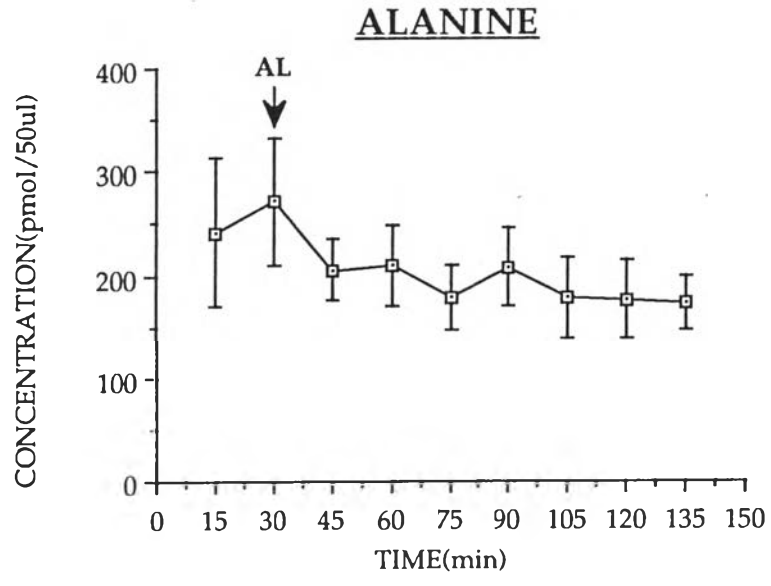


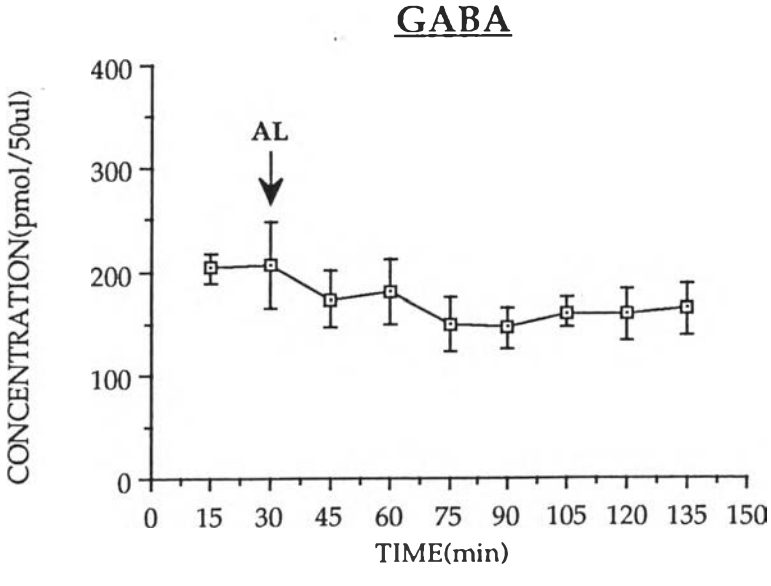
GLUTAMINE




GLYCINE
CONCENTRATION(pmole/50ul)
6000
4000
2000
0
AL
0 15 30 45 60 75 90 105 120 135 150
TIME(min)


TAURINE
CONCENTRATION(pmol/50ul)
1500
1200
900
600
300
0
AL
0 15 30 45 60 75 90 105 120 135 150
TIME(min)


ALANINE
CONCENTRATION(pmol/50ul)
400
300
200
100
0
AL
0 15 30 45 60 75 90 105 120 135 150
TIME(min)


GABA
CONCENTRATION(pmol/50ul)
400
300
200
100
0
AL
0 15 30 45 60 75 90 105 120 135 150
TIME(min)

TIME(min)	15	30	45	60	75	90	105	120	135
COMP.	CONTROL		UNILATERAL NERVE LESION						
ASP	891.36 ±47	909.41 ±49	936.71 ±63	922.95 ±72	1035.99 ±20	943.51 ±144	761.17 ±220	806.68 ±160	611.24 ±211
GLU	990.71 ±57	1073.66 ±116	975.75 ±210	799.99 ±197	815.29 ±235	713.58 ±291	595.27 ±280	551.92 ±235	569.71 ±218
SER	7177.68±1487	6016.43±1084	6971.89±1255	8006.97±1489	7213.65±1473	8882.09±474	7986.51±1523	5906.88±1556	6866.35±1184
GLN	1026.51±196	1224.49±170	1215.90±63	1103.07±133	1089.80±121	869.07±134	982.05 ±70	1037.42 ±124	972.04 ±46
GLY	3960.51±935	3532.24±713	3135.65±1025	2994.04±967	2946.04±967	2946.88±922	3168.96±826	3283.90±720	3629.04±922
TAU	1021.19±141	946.98±91	780.01±32	660.55±110	535.34±91	503.38±15	448.10 ±17	436.67 ±13	494.27 ±13
ALA	241.50±72	270.76 ±61	204.69 ±30	209.39 ±39	177.44 ±31	207.66 ±37	177.83 ±40	175.56 ±38	172.34 ±27
GABA	202.67 ±15	206.81 ±41	173.33 ±27	180.23 ±31	149.78 ±26	145.21 ±20	160.16 ±15	158.39 ±25	164.29 ±25

Table 5. Effect of unilateral electrolytical acute lesion of vestibular nerve on release of endogeneous amino acids. Data are expressed as mean (pmol/50 µl) ± S.E.M.

contralateral (CONTRA.) and lesioned (LES-3) vestibular nuclei. The amount of all amino acids release in the lesioned sides were lower than those of the contralateral sides. The level of glutamate, glutamine, taurine and alanine in the lesioned sides were significantly different at $p<0.05$ comparing with those in the contralateral sides (Fig.17 and Table 6).

In 7 days post lesion group, recovery from locomotor ataxia was observed. Movement of the rats were almost identical to those from normals. In microdialysis experiment, the samples were collected in the lesioned vestibular nuclei following with the contralateral vestibular nuclei by using the same probe. Figure 20 shows the pattern of release of endogenous amino acids from contralateral (CONTRA) and lesioned (LES-7) vestibular nuclei. In lesioned side, the level of glutamate showed the greatest decrease while the others were slightly lower than contralateral side except aspartate which the release in lesioned side was slightly higher than the contralateral side (Fig.21 and Table 7). However, there were no significantly different in all amino acids release between lesioned and contralateral side.

5. Effect of High-K^+ Solution on Amino Acids Release.

These experiments were carried out in order to compare the effect of the KCl-evoked release in 3 days post lesion rats (n=4) and normal rats (n=5). After the baseline release was established, usually after collection of two spontaneous release, dialysis was changed from normal aCSF to high K^+ (100 mM) solution for 30 min. After that the perfusion was changed back to

Figure 18. Chromatogram obtained from the analysis of perfusate fractions collected *in vivo* in the vestibular nuclei of 3 days post-lesion rats. All vestibular nerve lesions were performed on the left side. The samples were collected in the lesioned sides before collecting in the contralateral sides by using the same probe. Chromatogram 'LES-3' represents the release pattern in the 8th perfusate sample obtained from the left vestibular nuclei, whereas chromatogram 'CONTRA' represents pattern in the 8th perfusate sample obtained from the right vestibular nuclei of the same lesioned rats.

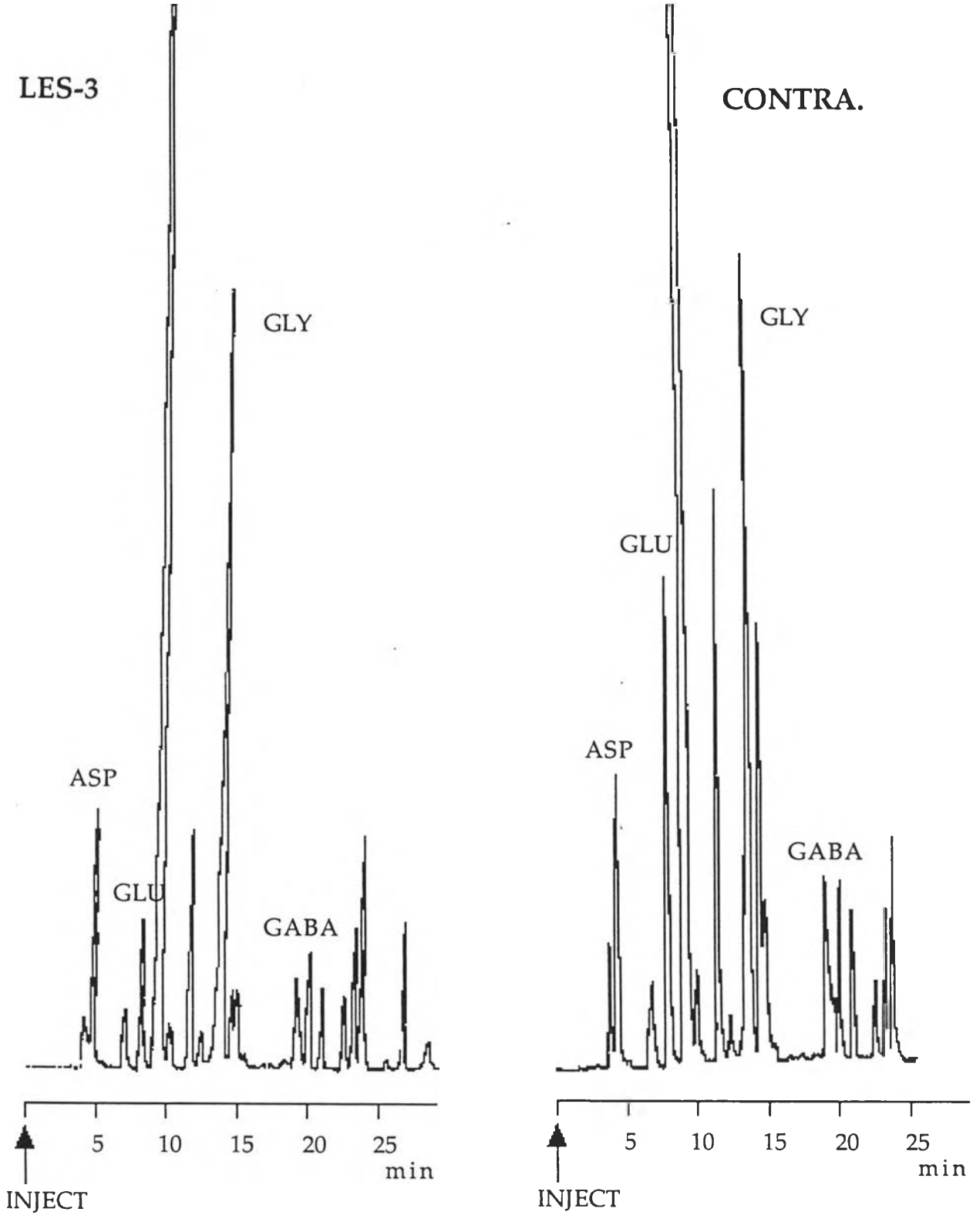
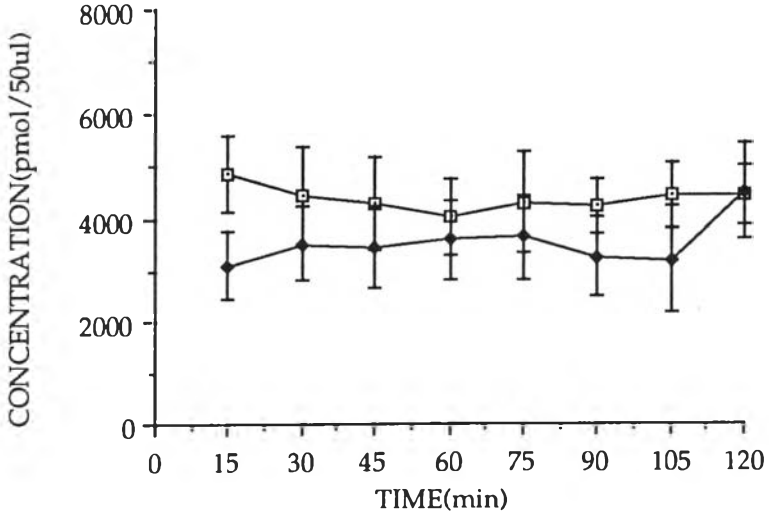

LES-3
GLY
ASP
GLU
GABA
5
10
15
20
25
min
INJECT
CONTRA.
GLY
GLU
ASP
GABA
5
10
15
20
25
min
INJECT

Figure 19. Level of each amino acid in the left vestibular nuclei of 3 days post-lesion rats compared with those release in the right vestibular nuclei. All vestibular nerve lesion were performed on the left side (n=5). The samples were collected in the lesioned sides following with the contralateral sides by using the same probe. The sample Amino acids examined were Asp, Glu, Ser, Gln, Gly, Tau, Ala and GABA. Values are expressed as mean (pmol/50µl) and S.E.M. is shown by the vertical bar. n = number of observations. * $p<0.05$, Student 's paired t-test.

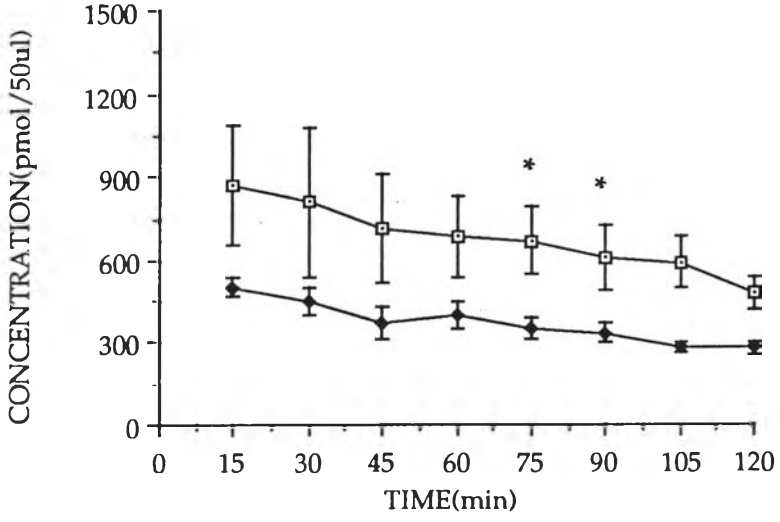
◆——◆ represent data for each amino acid in lesioned sides.

▫——▫ represent data for each amino acid in contralateral sides.

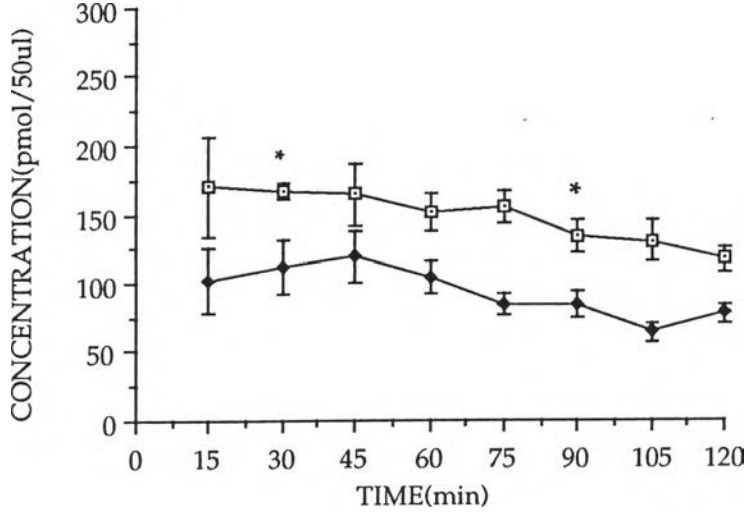
GLYCINE



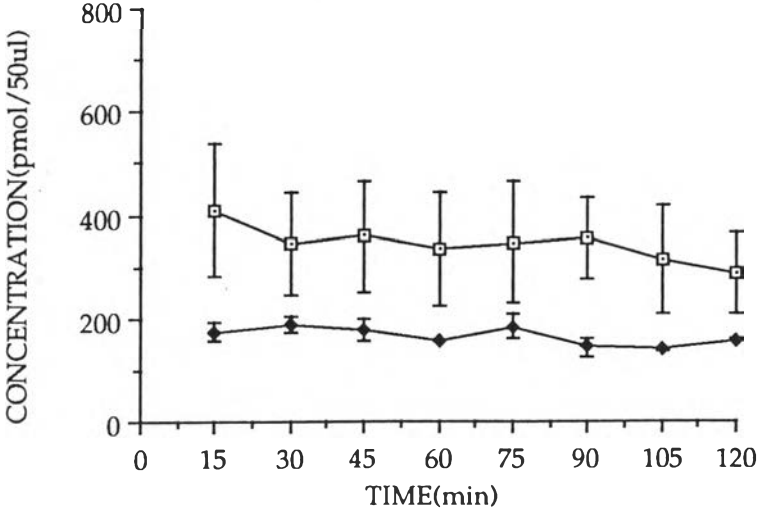
TAURINE



ALANINE



GABA



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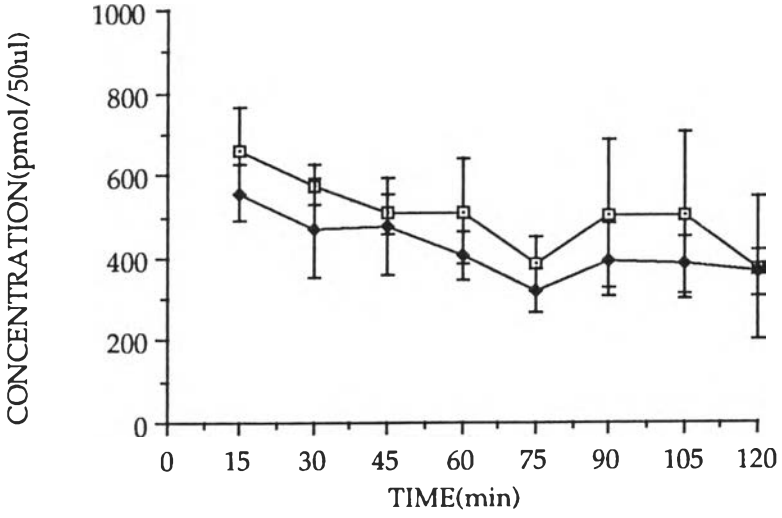
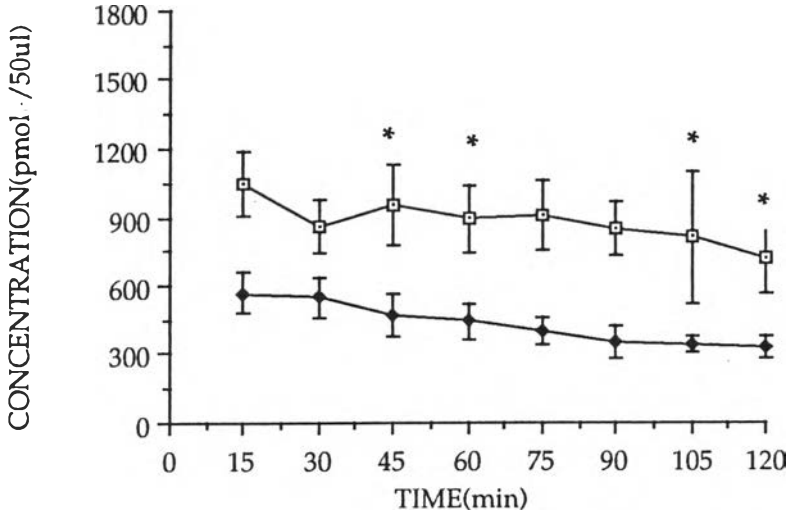
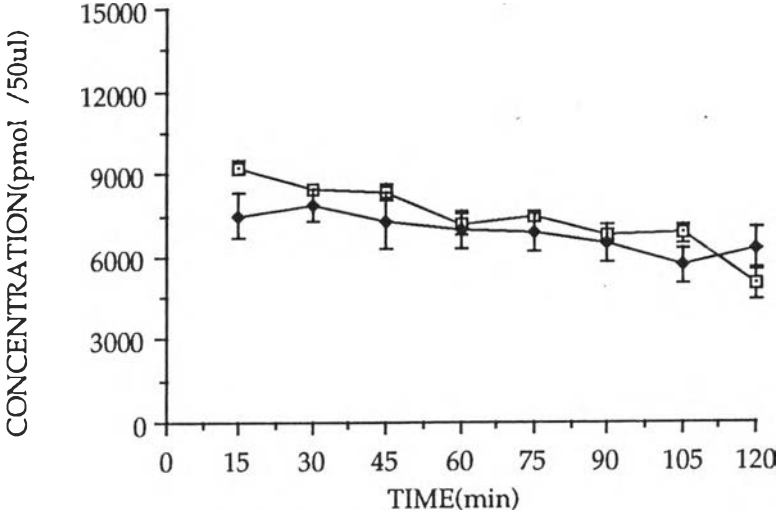


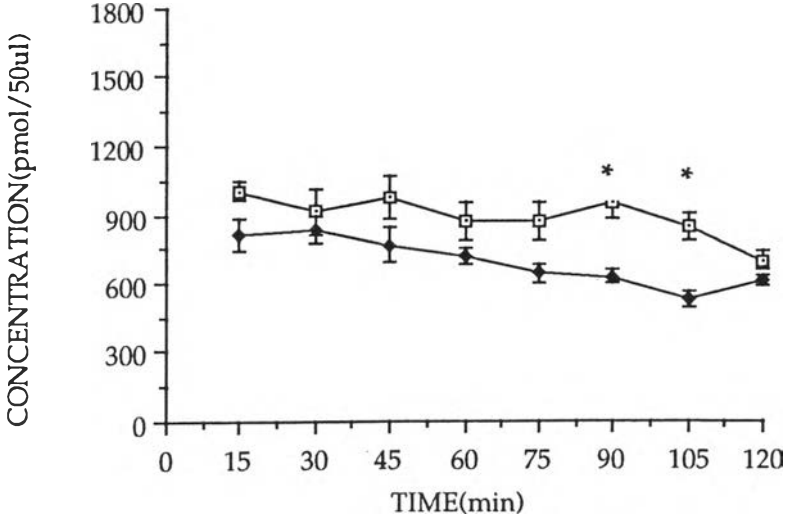
GLUTAMATE



SERINE



GLUTAMINE



TIME (MIN)	15	30	45	60	75	90	105	120
COMP.	aCSF							
ASP CONT.	661.04±102	575.93±49	507.26±48	511.84±128	386.73±67	505.42±178	504.29±204	375.12±173
LESION	558.03±69	473.76±118	474.73±117	405.40±58	320.10±52	393.77±89	382.95±67	362.92±55
GLU CONT.	1047.95±139	858.06±116	951.56±175	891.45±145	904.46±148	844.42±116	808.00±291	718.41±149
LESION	569.67±85	547.80±89	470.57±93*	442.01±79*	396.15±58	357.89±71	338.27±32*	332.94±45*
SER CONT.	9258.67±212	8456.80±5	8312.39±291	7201.39±420	7419.53±126	6797.10±189	6845.90±347	4968.7±577
LESION	7497.44±813	7811.93±604	7275.8±1008	6926.08±670	6845.44±663	6513.88±683	5670.7±622	6257.42±799
GLN CONT.	1003.33±43	913.09±101	977.22±96	871.80±78	872.19±82	955.48±69	847.79±55	699.79±44
LESION	813.35±75	833.33±57	770.31±75	713.54±36	641.89±38	627.68±29*	531.34±37*	613.09±25
GLY CONT.	4873.82±725	4458.70±942	4279.92±885	4014.67±724	4311.84±991	4231.82±526	4453.68±638	4432.54±576
LESION	3093.04±660	3527.09±712	3459.35±790	3582.38±777	3651.25±803	3260.32±747	3210.5±1019	4514.80±922
TAU CONT.	873.44±216	810.14±269	716.79±195	686.83±149	668.38±123	607.62±115	589.51±94	481.60±58
LESION	504.52±36	453.24±54	371.00±58	401.99±49	353.61±38*	334.85±34*	281.36±18	280.71±27
ALA CONT.	169.72±35	167.36±5	164.10±22	151.65±13	155.04±11	132.94±12	129.84±14	116.92±9
LESION	101.46±23	112.55±19*	118.63±18	103.72±12	85.13±7	84.60±10*	63.94±7	78.17±6
GABA CONT.	409.75±128	345.11±97	358.29±106	335.57±110	346.17±117	357.64±78	314.72±103	288.74±78
LESION	174.81±16	188.90±13	178.49±20	156.83±2	185.54±23	144.25±19	139.53±2	158.34±3

Table 6. Effects of 3 days post unilateral lesion of vestibular nerve on release of endogeneous amino acids. Data are expressed as mean±S.E.M

* Statistically different from control side , $p<0.05$(Student's paired t-test)

Figure 20. Chromatogram obtained from the analysis of perfusate fractions collected *in vivo* in the vestibular nuclei of 7 days-post lesion rats. All vestibular nerve lesion were performed on the left side. The samples were collected in the lesioned sides following with the contralateral sides by using the same probe. Chromatogram 'LES-7' represents the release pattern in the 4th perfusate sample obtained from the left vestibular nuclei, whereas chromatogram 'CONTRA' represents pattern in the 4th perfusate sample obtained from the right vestibular nuclei of the same lesioned rats.

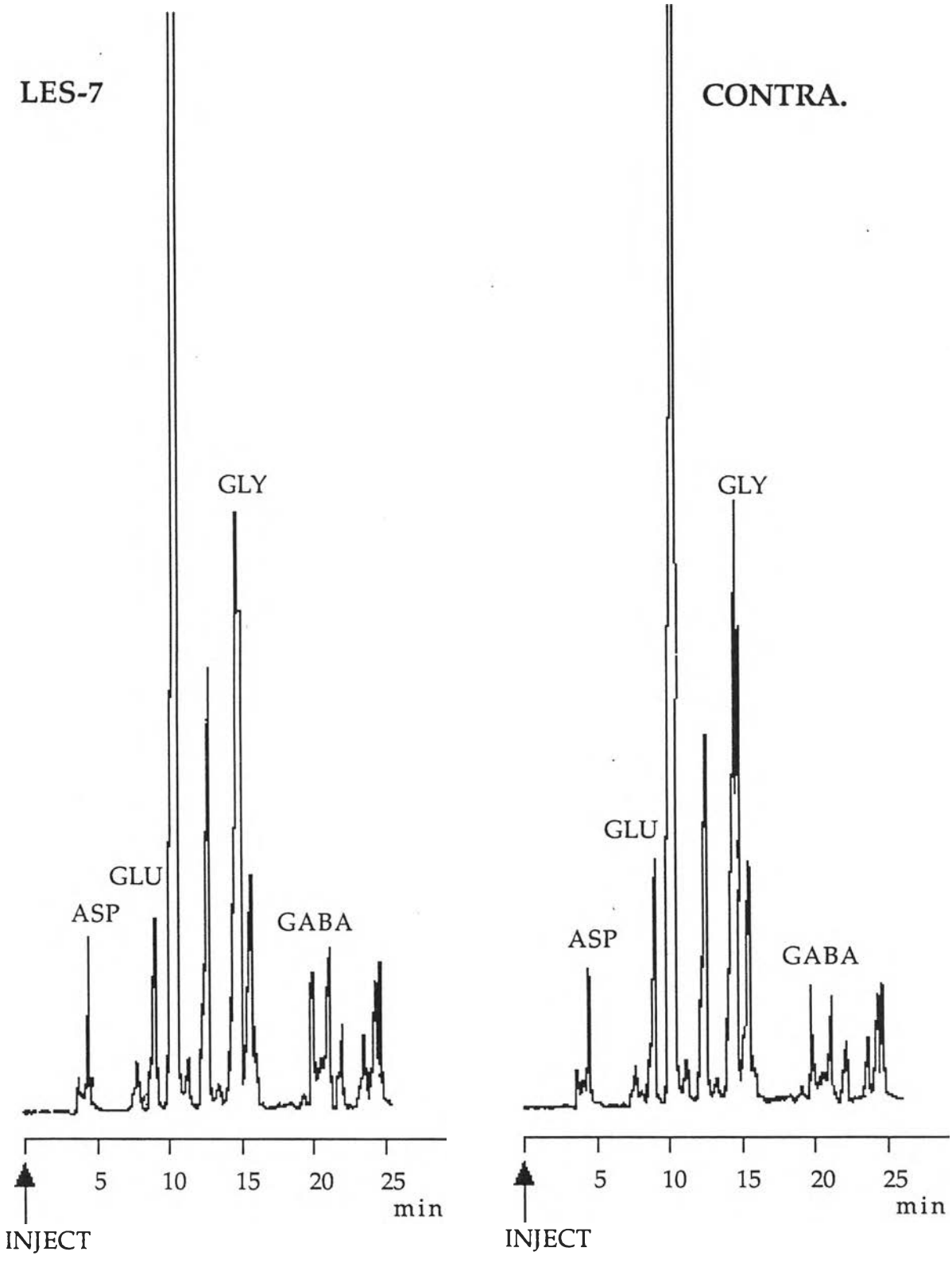
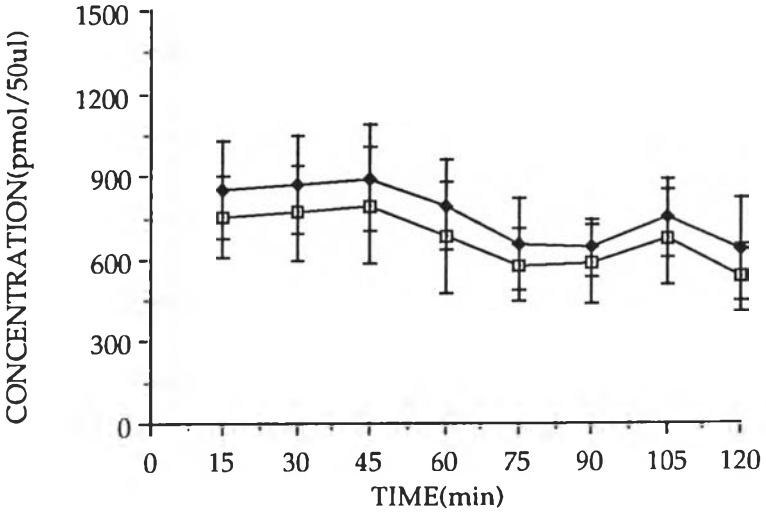

LES-7
GLY
GLU
ASP
GABA
5
10
15
20
25
min
INJECT
CONTRA.
GLY
GLU
ASP
GABA
5
10
15
20
25
min
INJECT

Figure 21. Level of each amino acid in the left vestibular nuclei of 7 days post-lesioned rats compared with those release in the right vestibular nuclei. All vestibular nerve lesions were performed on the left side (n=5). The samples were collected in the lesioned sides following with the contralateral sides by using the same probe. Amino acids examined were Asp, Glu, Ser, Gln, Gly, Tau, Ala and GABA. Values are expressed as mean (pmol/50µl) and S.E.M is shown by the vertical bar. n = number of observations. * $p<0.05$, Student 's paired t-test.

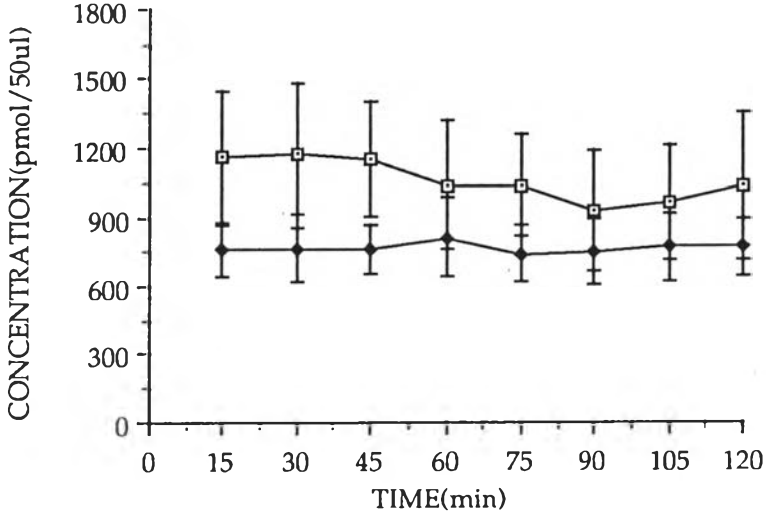
●——● represent data for each amino acid in lesioned sides.

▫——▫ represent data for each amino acid in contralateral sides.

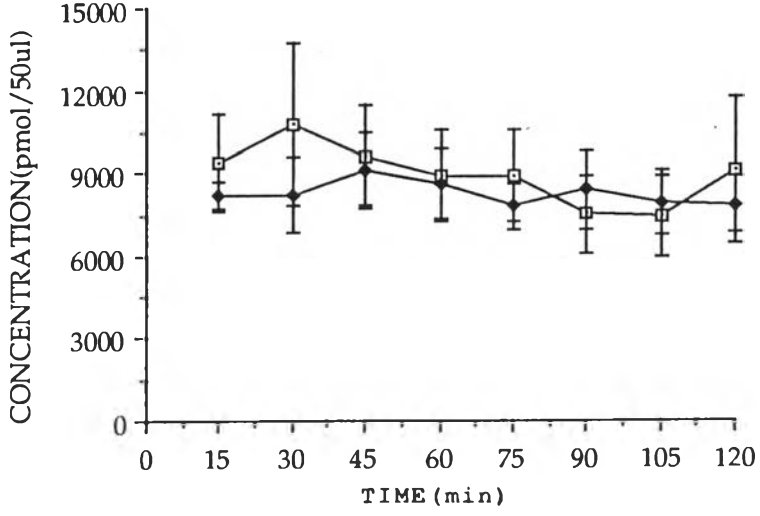
ASPARTATE



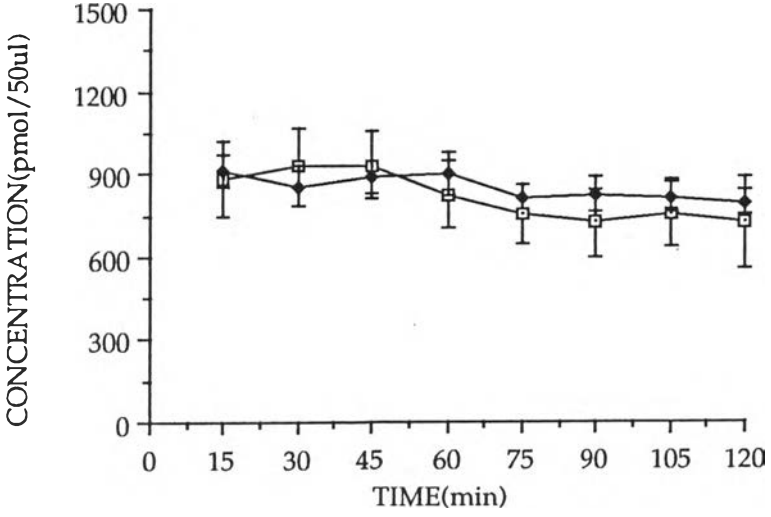
GLUTAMATE



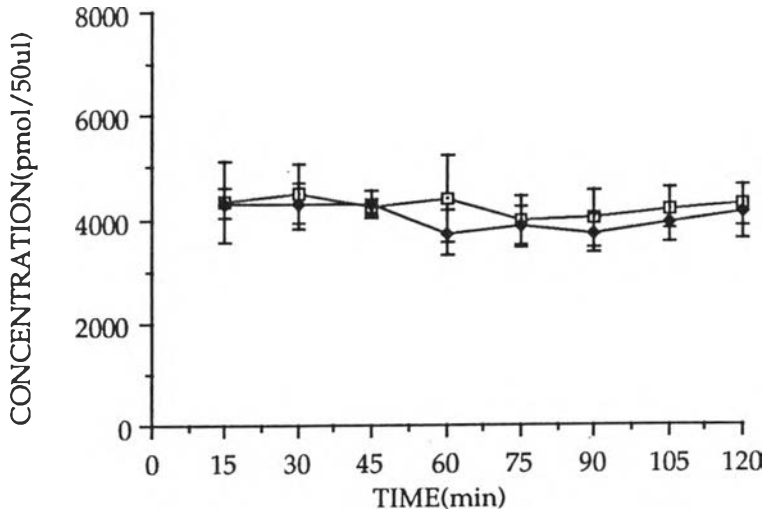
SERINE



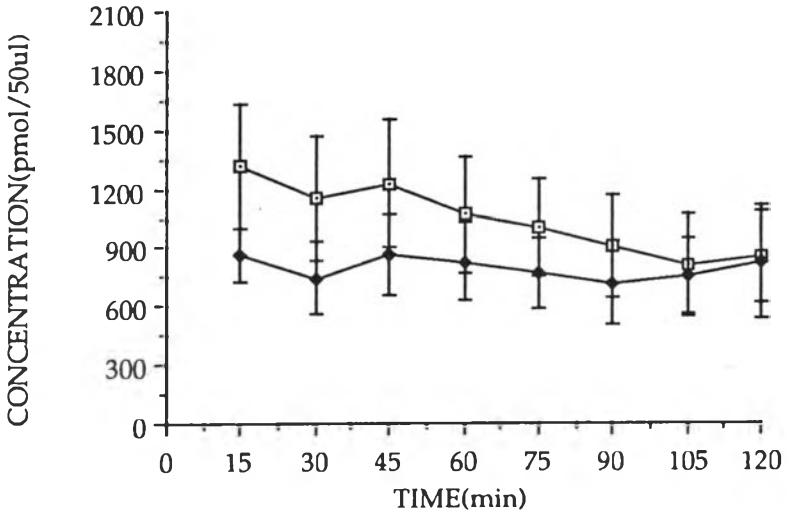
GLUTAMINE



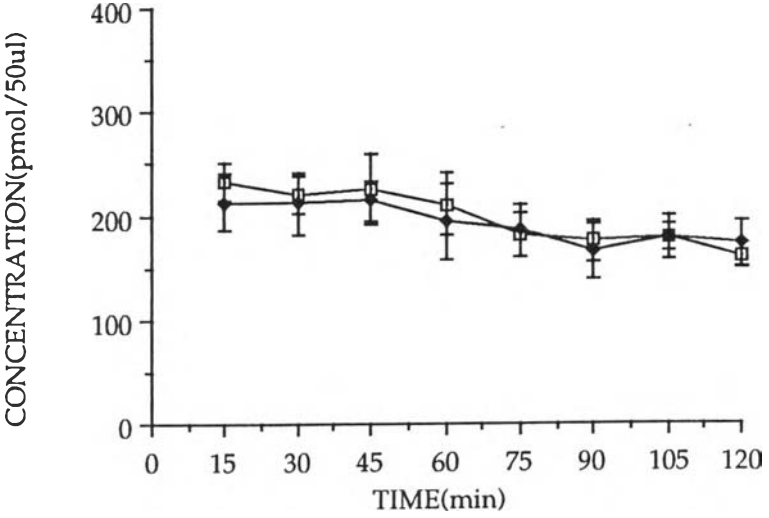
GLYCINE



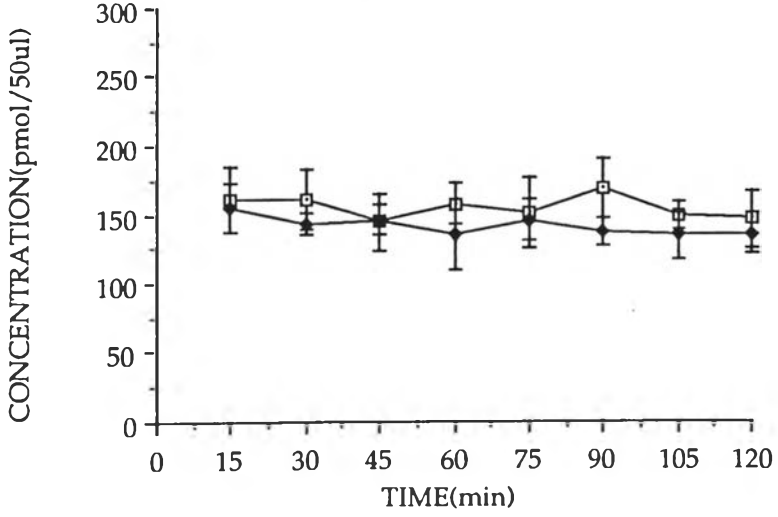
TAURINE



ALANINE



GABA



TIME (MIN)	15	30	45	60	75	90	105	120
COMP.	aCSF							
ASP CONT.	751.89±147	771.49±171	795.21±209	682.18±203	580.72±134	583.49±138	679.19±173	536.76±124
LESION	853.12±172	874.97±177	894.63±189	795.56±161	656.41±169	642.83±103	754.11±142	638.07±182
GLU CONT.	1160.46±286	1170.92±312	1154.75±245	1040.13±272	1037.56±218	929.23±253	965.50±250	1032.07±3117
LESION	726.34±119	767.73±144	761.43±1108	816.44±169	743.19±121	752.03±1137	772.65±146	771.15±123
SER CONT.	9416.7±1805	10782±2929	9577.6±1861	8932.0±1681	8883.2±1658	7502.5±1468	7453.7±1479	9112.5±2630
LESION	8242.4±477	8204.0±1356	9143.9±1338	8626.8±1268	7822.8±840	8392.9±1409	7938.7±1212	7867.9±1037
GLN CONT.	884.07±139	927.41±139	933.47±121	825.12±124	756.07±110	724.54±124	758.28±125	728.93±169
LESION	913.70±58	857.02±73	892.39±56	905.63±80	815.42±45	824.05±64	818.18±58	796.06±44
GLY CONT.	4347.8±770	4511.4±576	4221.7±162	4394.7±826	3966.8±479	4016.8±547	4203.6±382	4273.0±379
LESION	4301.5±288	4268.6±446	4293.9±278	3732.0±462	3846.1±369	3714.3±364	3914.6±364	4141.2±512
TAU CONT.	1322.27±315	1150.85±320	1225.80±324	1064.13±294	999.98±249	904.50±262	810.95±265	854.98±232
LESION	864.99±135	747.93±186	869.33±205	829.41±203	766.46±181	720.18±212	754.42±189	822.64±283
ALA CONT.	232.01±18	219.16±18	224.79±34	210.08±29	180.35±19	174.87±13	177.87±13	160.42±12
LESION	212.99±27	210.56±29	213.35±19	194.03±36	184.64±25	165.05±25	178.25±20	171.37±21
GABA CONT.	160.80±23	160.38±21	144.48±20	157.57±14	151.34±25	168.63±21	149.03±9	146.19±20
LESION	155.79±17	142.38±7	145.96±11	135.44±24	145.88±15	137.41±10	134.62±16	135.82±14

Table 7. Effects of 7 days post unilateral electrolytical lesion of vestibular nerve on release of endogenous amino acids . Data are expressed as mean(pmol/50µl)± S.E.M

Figure 22. Chromatogram obtained from the analysis of perfusate fractions collected *in vivo* in the vestibular nuclei in normal rats by a microdialysis technique. Those peaks corresponding to the OPA-derivatives of amino acids, identified by comparison of their retention time with those of standard components, have been indicated in the Fig.9. Chromatogram 'CONTROL' represents the release pattern in the 2nd sample fraction obtained during a control situation, whereas chromatogram 'HIGH K^+ STIMULATION' represents the release pattern in the 6th sample fraction obtained after replace aCSF with high-K^+ (100 mM) solution.

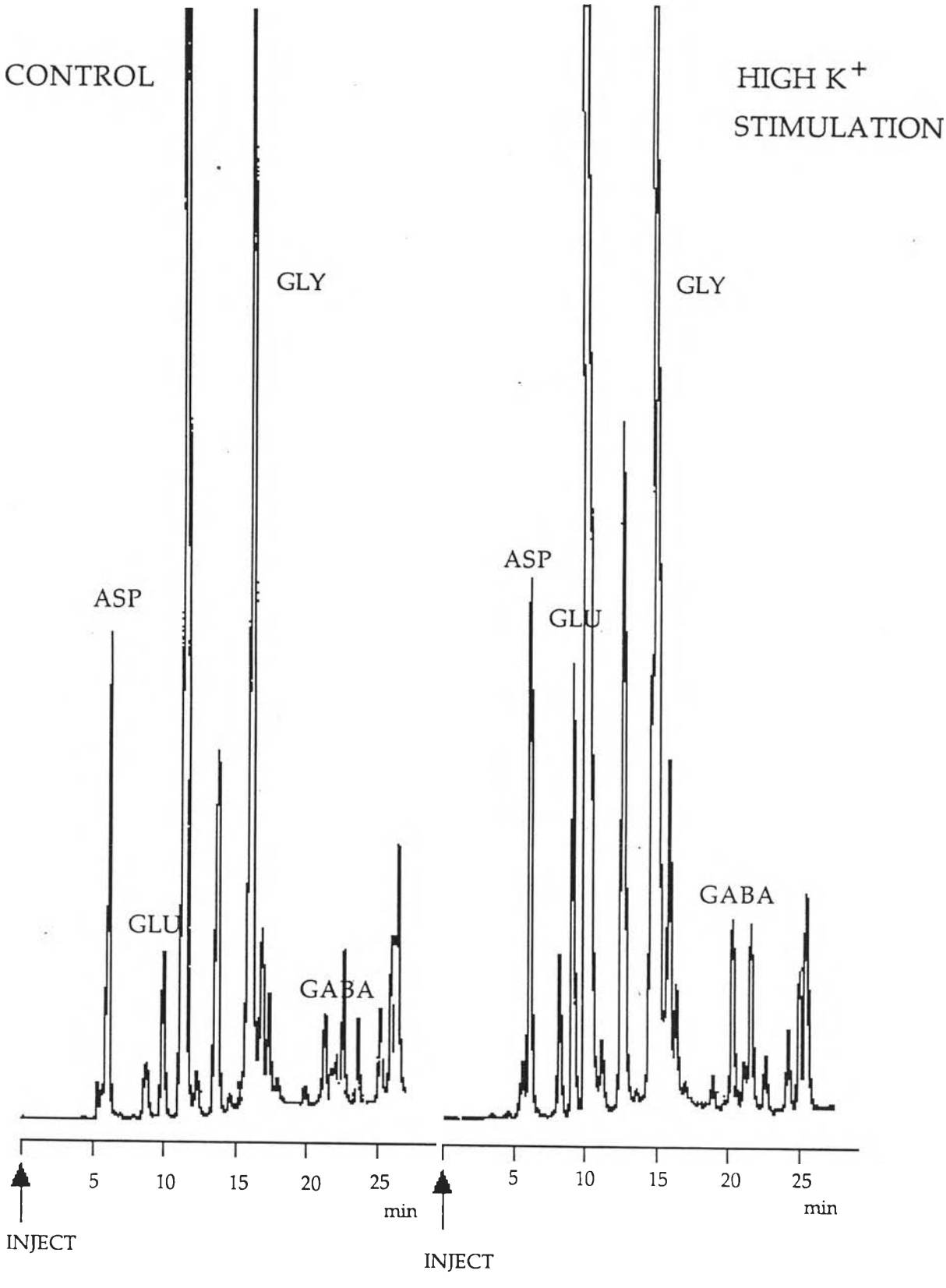
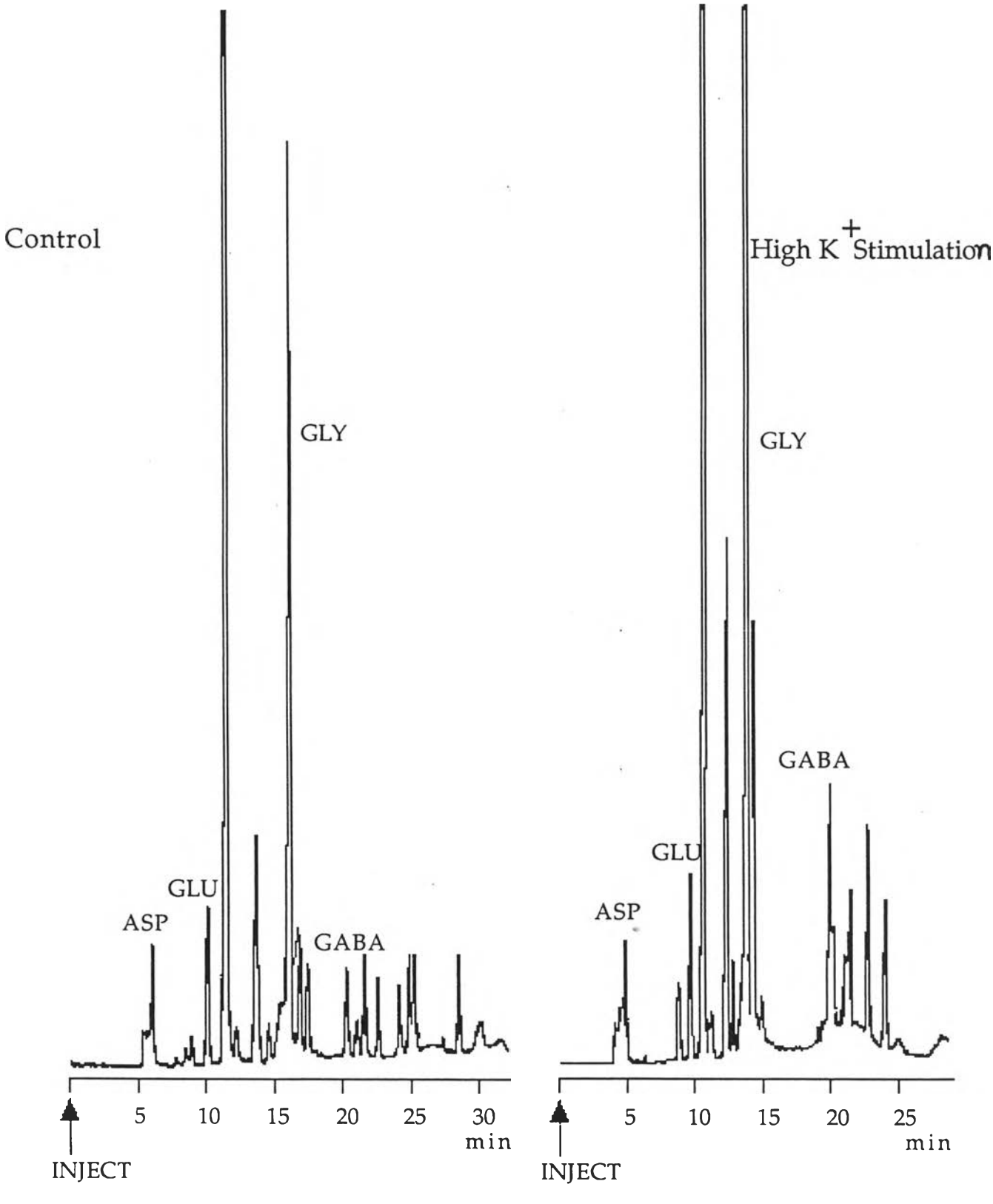

CONTROL
HIGH K+
STIMULATION
GLY
GLY
ASP
ASP
GLU
GLU
GABA
GABA
5
10
15
20
25
min
5
10
15
20
25
min
INJECT
INJECT

Figure 23. Chromatogram obtained from the analysis of perfusate fractions collected *in vivo* in the vestibular nuclei in 3 days post-lesion rats by a microdialysis technique. Those peaks corresponding to the OPA-derivatives of amino acids, identified by comparison of their retention times with those of standard components, have been indicated in the Fig.9. Chromatogram 'CONTROL' represents the release pattern in the 2nd perfusate fraction obtained during a control situation, Whereas chromatogram 'HIGH K^+ STIMULATION' represents the release pattern in the 6th perfusate sample obtained after replace aCSF with high-K^+ (100mM) solution.


Control
GLY
GLU
ASP
GABA
5
10
15
20
25
30
min
INJECT
High K+ Stimulation
GLY
GLU
ASP
GABA
5
10
15
20
25
min
INJECT

normal aCSF. Figure 22 and 23 show the pattern of release of endogenous amino acids from control and after high K^+ stimulation in normal and 3 days lesion rats respectively. In normal rats, replacing the normal aCSF with high K^+ solution led to a marked overflow of aspartate, taurine and glutamate with significantly different from those of the control ($p< 0.005$, $p<0.05$ and $p<0.01$ for Asp, Tau, and Glu respectively) whereas the release of serine, glutamine, glycine, alanine and GABA also increased but not significantly. By contrast, in lesion rats only aspartate and glycine showed a significant increase from control at $p<0.05$ and $p<0.005$ respectively while the others showed only a slight increase after high K^+ stimulation (Fig.24 and Table 8, 9). Table 10 illustrates the comparison of % increase of each amino acids after high K^+ evoked release in normal rats and 3 day post lesion rats. It was found that % difference of glutamate release between two groups was greater than the others.

Figure 24. Comparion of effects of KCl-evoked release of each amino acids in the left vestibular nuclei of 3 days post-lesion rats (n=4) and normal rats (n=5). The initial superfusion medium was the normal aCSF. After the second sample was collected, the medium was replaced with high K+ (100 mM) for 30 min. Amino acids examined were Asp, Glu, Ser, Gln, Gly, Tau, Ala and GABA. Each point represents the mean release of amino acid (pmol/50µl) and S.E.M. is shown by the vertical bar.

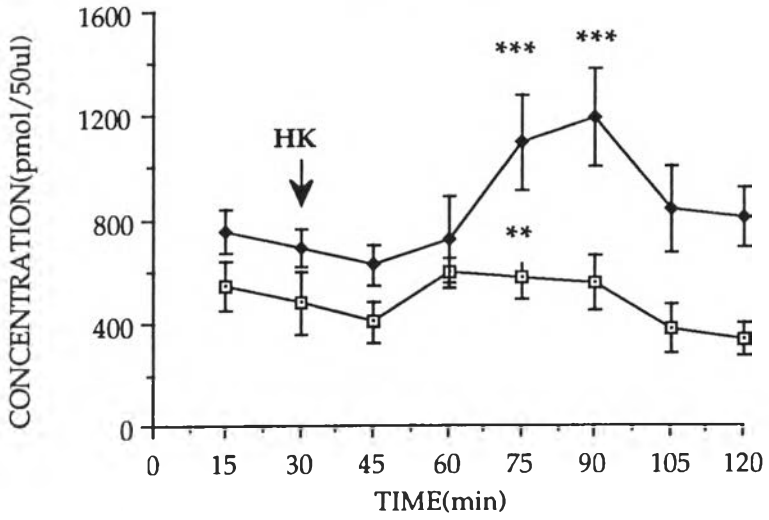
n = number of observations.

* $p < 0.01$, ** $p < 0.05$, *** $p < 0.005$, Student's t-test.

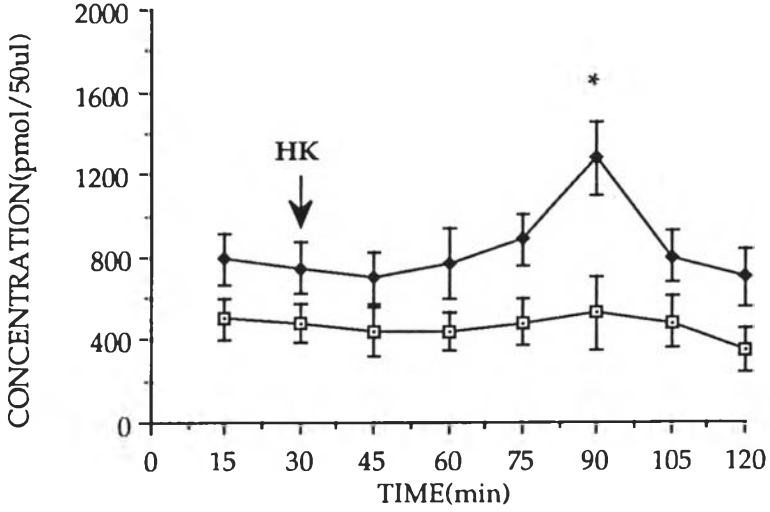
◆——◆ represent data for each amino acid from KCl-evoked release of normal rats.

□——□ represent data for each amino acid from KCl-evoked release of lesioned rats.

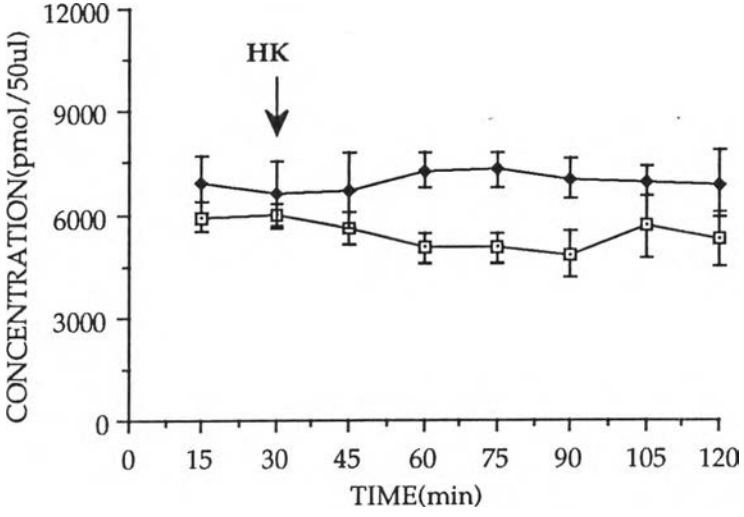
ASPARTATE



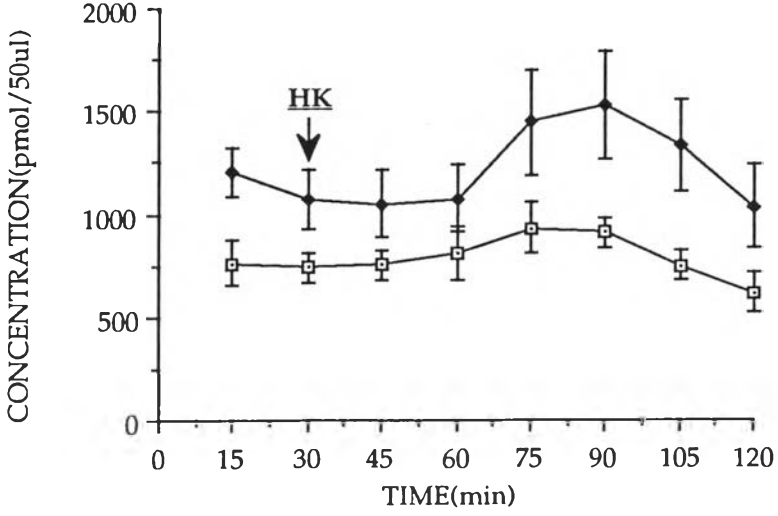
GLUTAMATE

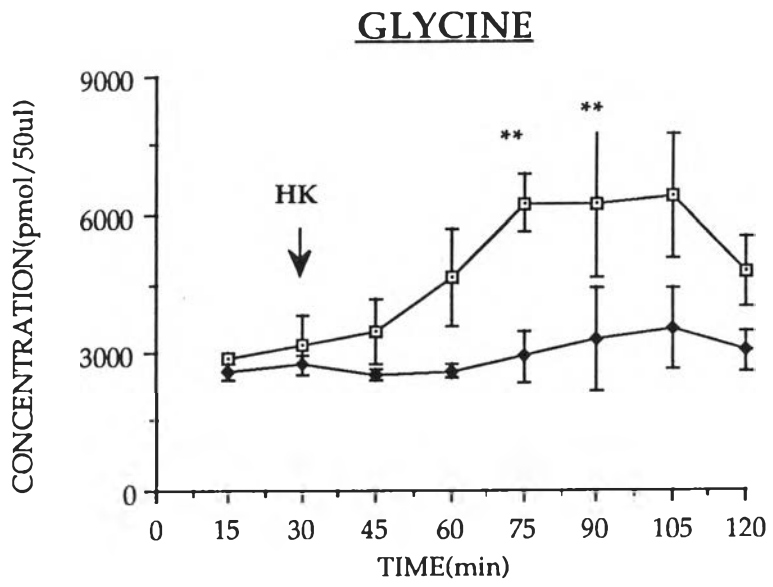


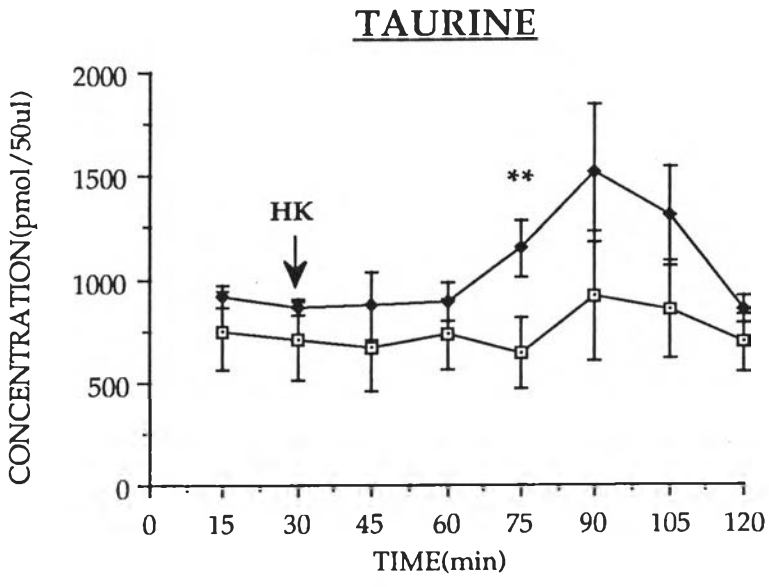
SERINE

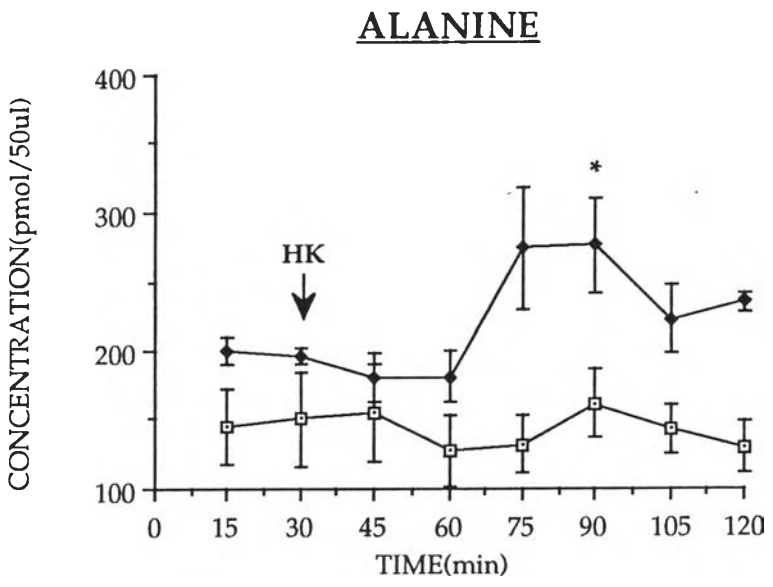


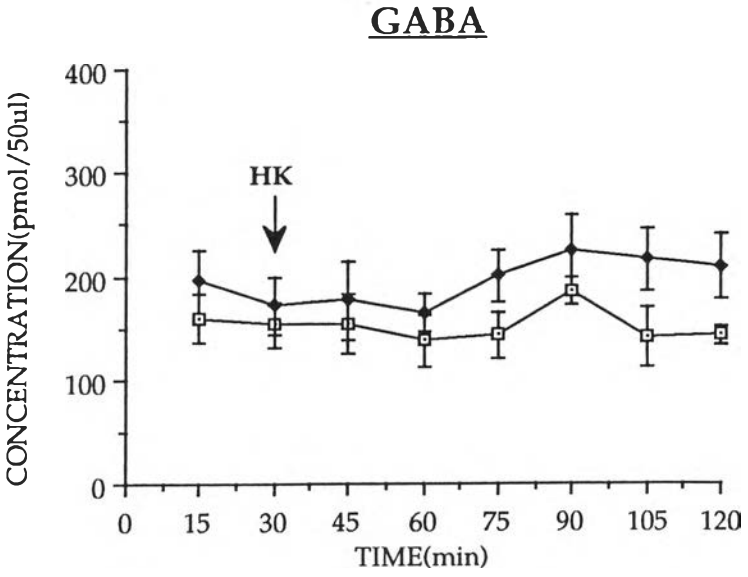
GLUTAMINE




GLYCINE
CONCENTRATION(pmol/50ul)
9000
6000
3000
0
HK
**
**
0 15 30 45 60 75 90 105 120
TIME(min)


TAURINE
CONCENTRATION(pmol/50ul)
2000
1500
1000
500
0
HK
**
0 15 30 45 60 75 90 105 120
TIME(min)


ALANINE
CONCENTRATION(pmol/50ul)
400
300
200
100
HK
*
0 15 30 45 60 75 90 105 120
TIME(min)


GABA
CONCENTRATION(pmol/50ul)
400
300
200
100
0
HK
0 15 30 45 60 75 90 105 120
TIME(min)

TIME(min)	15	30	45	60	75	90	105	120
COMP.	aCSF		HIGH K+		aCSF			
ASP	754.99 ±86	690.72 ±76	623.30 ±77	722.11 ±164	1095±181***	1190±188***	835.77 ±164	805.21 ±117
GLU	792.11 ±129	748.94 ±127	702.33 ±122	771.35 ±173	884.09 ±128	1278.82±176*	802.73 ±124	700.06 ±140
SER	6863.14±846	6549.34±981	6663.49±1062	7252.43±541	7267.02±504	7007.33±610	6906.89 ±431	6855.4±1010
GLN	1199.80±121	1071.11±140	1051.85±168	1077.94±163	1449.99±255	1526.94±265	1331.43 ±225	1037.10 ±205
GLY	2594.71±178	2740.82±192	2535.77±140	2602.48±138	2912.7±584	3297±1138	3531.1±909	3044.02±454
TAU	912.79 ±48	856.51 ±32	872.17 ±161	886.44 ±90	1146.2±137**	1511.26±337	1301.42 ±246	850.74 ±69
ALA	200.42 ±10	195.45 ±6	180.14 ±18	181.29 ±19	273.68 ±44	275.75 ±35	222.52 ±25	235.13 ±7
GABA	194.97 ±30	171.80 ±27	176.63 ±37	164.22 ±18	200.37 ±24	224.76 ±34	215.93 ±31	209.34 ±31

Table 8. Evoked release of endogenous amino acids in the vestibular nuclei of normal group by stimulated with high k+ (100 mM). Data are expressed as mean (pmol/50µl)±S.E.M.

* Significantly different from control *p<0.01, ** p<0.05, *** p<0.005, Student's t-test.

TIME(min)	15	30	45	60	75	90	105	120
COMP.	aCSF		HIGH K+		aCSF			
ASP	543.51 ±97	477.61 ±121	406.06 ±78	593.99 ±58	576.47±89**	551.41 ±106	374.89 ±95	334.05 ±65
GLU	504.59 ±97	483.02 ±95	441.15 ±120	443.73 ±96	488.31 ±111	531.33 ±173	489.27 ±129	352.00 ±107
SER	5908.42±443	5974.58±290	5569.00 435	4993.35 ±448	4995.61 ±431	4808.29 ±685	5618.33 ±901	5241.18 ±785
GLN	764.69 ±112	743.74 ±71	751.89 ±77	812.68 ±131	934.51 ±118	910.12 ±67	748.23 ±75	619.12 ±97
GLY	2885.71±114	3180.36±635	3480.13±708	4633.1±1050	6249±630***	6231±1610**	6436.03±1348	4744.14±766
TAU	751.16 ±188	702.57 ±199	665.05 ±205	732.60 ±170	639.62 ±174	917.04 ±315	852.07 ±237	689.14 ±137
ALA	144.69 ±27	150.42 ±34	154.97 ±35	127.59 ±25	132.14 ±21	161.42 ±25*	143.31 ±17	130.00 ±19
GABA	159.84 ±24	153.06 ±23	154.51 ±28	138.27 ±25	142.61 ±22	186.84 ±13	140.30 ±29	143.35 ±9

Table 9. Evoked release of endogenous amino acids in the vestibular nuclei of 3 days post-lesion group by stimulated with high K^+ (100 mM). Data are expressed as mean (pmol/50µl)± S.E.M.
* Significantly different from control * $p<0.01$, ** $p<0.05$, *** $p<0.005$, Student's t-test.

Table 10. The comparison of % increase of each amino acid release after KCl- stimulation in normal and 3 days post-lesion rats.

Compound	%Increase of KCl-evoked release		%Difference
	in normal rats.	in lesioned rats.	
ASP	164.82	116.27	29.45
GLU	165.97	107.70	35.11
SER	108.37	84.08	12.74
GLN	134.45	124.04	7.74
GLY	132.40	206.10	-55.67
TAU	170.93	126.31	26.10
ALA	139.59	109.52	21.54
GABA	123.07	107.69	12.50