CHAPTER III

Results

Table 2 shows summary of iontophoretic study of A II on cortical neurones of the cerebellum.

Response of Purkinje Neurones to A II Administration

Satisfactory unitary recording were obtained from 96 cerebellar cortical neurones which were identified as Purkinje cells, 85 cells were sampled randomly from the cerebellar vermis while the others (11 cells) were sampled randomly from the cerebellar hemisphere.

In 80 of 85 vermian Purkinje cells (94.12 %) and in 11 hemisphere Purkinje cells (100 %) the effects of ionotophoretic application of A II (20 - 160 nA, positive current) were a 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 A II 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. For example, Figure 6 A shows the development of inhibition of neuronal firing of a Purkinje cell in the vermis during the application of A II at 70 nA, 80 nA and 100 nA respectively. That the observed response was an artefactual results of the current effects could be ruled out since application of positive current (carried by Na^+) from balancing channel of the microelectrode failed to produce any alternation in neuronal firing.

Table 2. Summary of iontophoretic study of A II on cortical neurones of the cerebellum.

	Number of neurones	
	Tested	Responsive
(a) Depressant action of A II		
(1) Purkinje cells of vermis	85	80
(2) Purkinje cells of hemisphere	11	11
(3) non-Purkinje cells	6	0
(b) A II induced enhancement of the actions		
(1) Depressants		
GABA	18	18
glycine	12	0
noradrenaline	5	0
taurine	5	0
5-hydroxytryptamine	6	0
(2) Excitants		
glutamate	4	0
aspartate	4	0
(c) Antagonism of the actions by saralasin		
A II (action on cell firing)	8	8
A II (enhancement of GABA action)	3	3
GABA	4	0
(d) Antagonism of the actions by bicuculline		
A II (action of cell firing)	5	5
A II (enhancement of GABA action)	3	3
GABA	5	5
glycine	4	0
5-hydroxytryptamine	2	0

Effects of Iontopheretic Application of A II on Unidentified Cortical Neurones (Non Purkinje Neurones)

In contrast, in 6 unidentified non Purkinje cells, iontophoretic application of A II with currents upto 120 nA failed to produce any change in the neuronal firing (Figure 6 B).

Effects of Iontophoretic Application of Saralasin

Saralasin, a specific A II antagonist, is a synthetic peptide which has the same structure as A II 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 A II. In the course of iontophoresis experiments, Phillip and Felix (1976) showed that the neuronal response to A II was totally inhibited during ejection of saralasin. In these experiment, saralasin was used as a tool for investigating the receptor mechanism of A II involement in depression of spontaneous Purkinje cell activity

Effect on the response to iontophoretically applied AII

Purkinje cells (n = 8) which were consistently depressed by iontophoretically administered A II (60 - 100 nA) were tested for the antagonistic activity of iontophoretic application of saralasin (positive current, 10 - 20 nA). In all cells tested, saralasin antagonized the depressant effect of a II by 60 - 100 % within 1 - 4 min. Example of this results is shown in Figure 7, in which it was shown that pulsatile iontophoretic application of A II (100 nA at 10 sec. periods) at regular intervals (10 sec.) produced consistent decrease in Purkinje cell discharge. During continuous saralasin application (10 nA), the A II induced depression of spontaneous

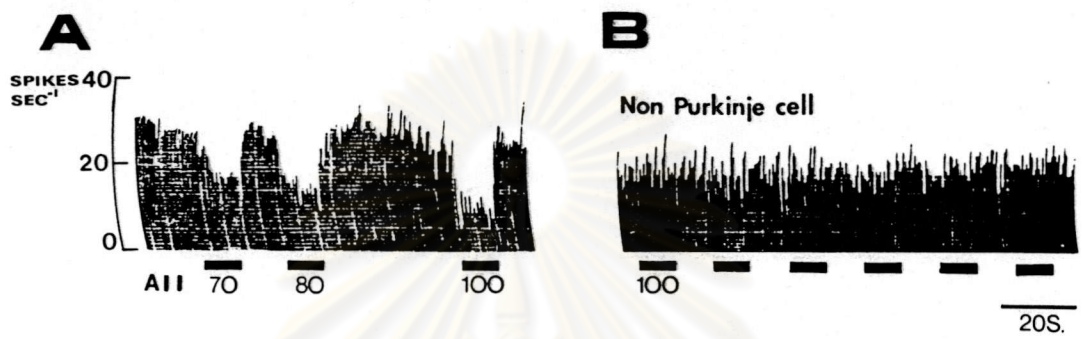

Figure 6. A and B Effects of iontophoretic administration of Angiotensin II (A II) on spontaneous firing of cerebellar neurone. A II produced firing depression on Purkinje cell in a dose dependent fashion (A), whereas in B, unidentified non-Purkinje cells did not response to the peptide. In this and 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 identical in both A and B.

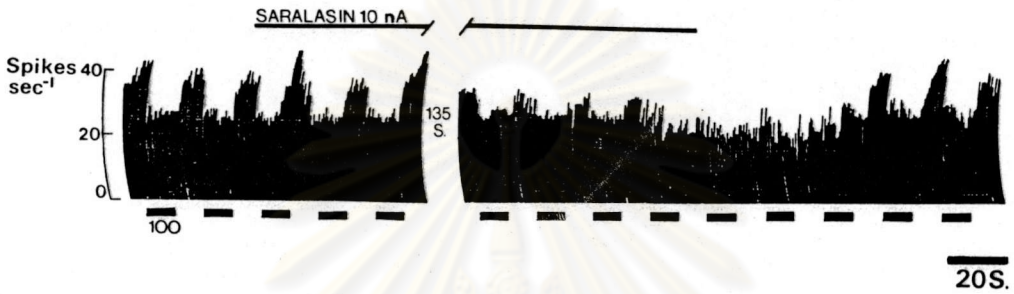

Figure 7. Effect of microiontophoretic administration of saralasin (10nA) on response of Purkinje cell to microiontophoretic A II (100 nA). The rate meter shows that 3 min after commencement of the application saralasin antagonized the depressant of A II, recovery to the control level of response was observed after cessation of saralasin administration.

discharge was remarkably reduced. Recovery to the control level of response was observed in each case after cessation of saralasin administration.

Note in this figure the saralasin alone also produced a mild depression of the cell firing.

The Purkinje cells (n = 4) which were consistently depressed by iontophoreticcally administered A II (60 - 90 nA) and GABA (5 - 20 nA) were tested for the antagonistic activity of iontophoretic application of saralasin (10 - 20 nA). In all cells tested, saralasin antagonized the depressant effect of A II by 60 - 90 % within 1 - 3 min, while the depression of neuronal activity caused by GABA remained unaffected. The rate meter record in Figure 8 shows response of Purkinje cell to pulsatile iontophoretic application of A II (60 nA for 10 sec) and GABA (10 nA positive current for 10 sec) at regular intervals (10 sec) before, during and after continuous iontophoretic application of saralasin. On this cell, saralasin showed a specific antagonism to the depressant effect of A II while the depressant effect of GABA remained unaffected. Recovery to the control level of response was observed in each case after cessation of saralasin administration.

Effect of A II on Neuronal Response to Putative Neurotransmitters (Selective Potentiation of GABA Induced Inhibition by A II).

Effect on the responses to iontophoretically applied depressant amino acids (GABA, glycine and taurine).

Pulsatile cationic iontophoretic applications (10 sec duration and at 10 sec regular intervals) of putative inhibitory amino acid neurotransmitters, GABA (5 - 40 nA, n = 18), GLY (20-100 nA, n =12)

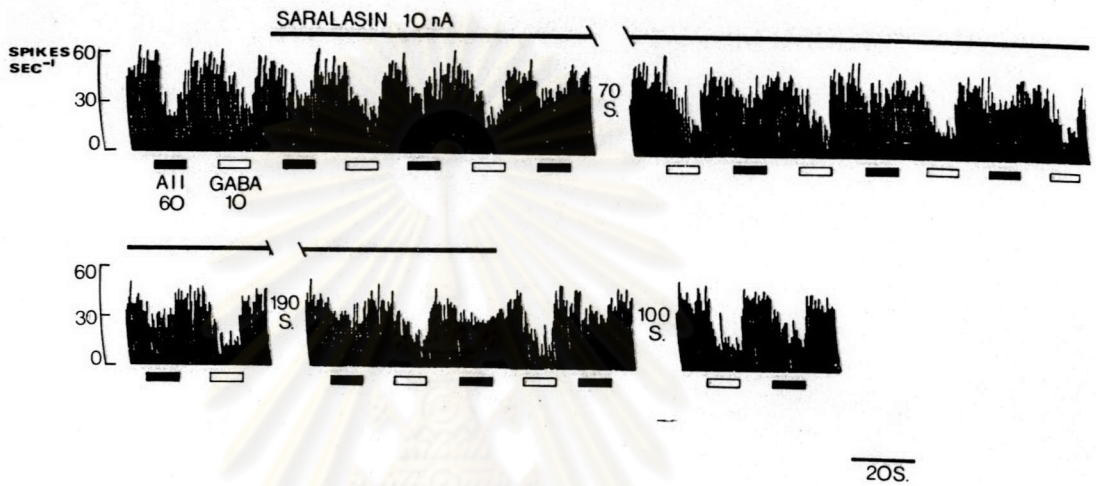

Figure 8. Effect of microiontophoretic administration of saralasin (10 nA) on responses of a Purkinje cell to iontophoretic application A II (60 nA) and GABA (10 nA)。 The rate meter shows that about 90 sec. after commencement of the application, saralasin selectively antagonized depressant action of A II, whereas the action of GABA was unaffected. Recovery to the control level of response was observed after cessation of saralasin administration.

and TAU (60 - 90 nA, n = 5) produced consistant depression of all Purkinje cell tested. When continuous microiontophoretic application of A II (20 - 80 nA) were superimposed on the depressant pulses, in all cells tested, the inhibitory response to GABA were selectively augmented during microiontophoretic application of A II, while the depression of neuronal activity caused by GLY and TAU remained unaffected. The onset of A II induced potentiation of GABA response was apparent within 1 - 4 min after commencement of A II application and this potentiation was progessive with time. The iontophoretic currents of A II in this case was adjusted to be subthreshold for producing depression of each neurone tested, so that depression induced by the peptide was considered minimal. Example of these results is shown in Figure 9, recovery to the control level of responses was observed in each case after cessation of A II administration.

Effect on the responses to iontophoretically applied monoamine transmitters (NA and 5-HT)

Pulsatile cationic iontophoretic applications (10 sec duration and at 10 sec regular intervals) of putative inhibitory amine neurotransmitters NA (20 - 110 nA, n = 5), 5-HT (50 - 90 nA, n = 6) and GABA (20 - 40 nA, n = 6) produced consistent depression of all Purkinje cells tested. When continuous microiontophoretic applications of A II (20 - 80 nA) were superimposed on the depressant pulses, in all cells tested, the depression of Purkinje cell activity caused by NA and 5-HT remained unaffected, while the inhibitory response to GABA were augmented during microiontophoretic application of A II. Figure 10 shows a clear example for this effect

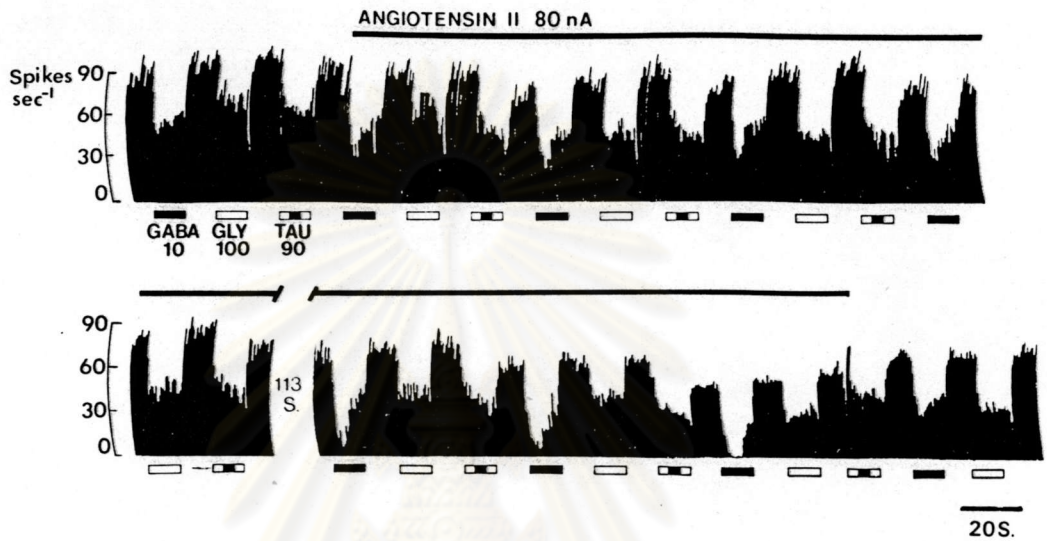

Figure 9. Effect of continuous iontophoretic application of angiotensin II (A II; 80 nA) on depressant actions of γ-aminobutyric acid (GABA; 10 nA), glycine (GLY; 100 nA) and taurine (TAU; 90 nA) on Purkinje cell. The rate meter shows that A II selectively potentiates inhibitory action of GABA without having any appreciable effects on actions of the other depressants. Recovery of the potentiation to the control level of response was observed after cessation of A II administration.

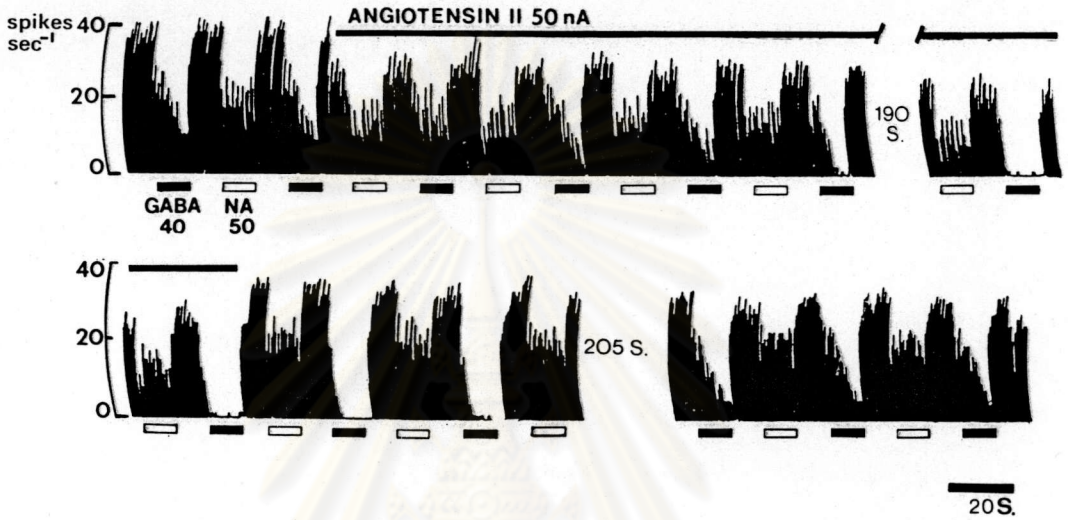

Figure 10. Effect of continuous application of angiotensin II (A II; 50 nA) on depressant action of γ -aminobutyric acid (GABA; 40 nA) and nor-adrenaline (NA; 50 nA) on Purkinje cell. The rate meter shows that A II selectively potentiates inhibitory action of GABA, while the depression of neuronal activity caused by NA remained unaffected. Note in this case that only partial recovery of the potentiation was observed.

were prolonged A II application induced a complete supression of Purkinje cell firing during application of GABA with the dose which otherwise, without A II application, produced approximately 50 per cent depression of spontaneous discharge. While pulsatile application of NA (50 nA, 10 sec) which produced approximately 50 per cent depression of the spontaneous discharge remained unaffected. Note in this case that a partial recovery of the potentiation was observed. Another example is also presented in Figure 11, in which it is showed that pulsatile iontophoretic applications of NA (110 nA, 10 sec), GABA (20 nA, 10 sec) and 5-HT (90 nA, 10 sec) at regular intervals (10 sec) produced approximately 20 per cent depression of spontaneous discharge. When continuous microiontophoretic application of A II (50 nA) was superimposed on depressant pulses, the inhibitory response to GABA were augmented (approximately to 70 per cent depression). After the termination of A II application, partial recovery was found in this neurone.

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

Tests were also advanced further to see whether there was any A II-induced modification of excitatory responses of Purkinje cell to putative excitatory neurontransmitter GLU and ASP. Pulsatile anionic iontophoretic application (10 sec duration and 10 sec regular intervals) of GLU (20 - 40 nA; n = 4) and ASP (25 - 40 nA; n = 4) produced consistent excitation in all Purkinje cells tested. When continuous microiontophoretic application of A II (80 - 100 nA) were superimposed on the excitant pulses, the excitation of Purkinje cells activity caused by GLU and ASP remained unaffected. No change in the neuronal

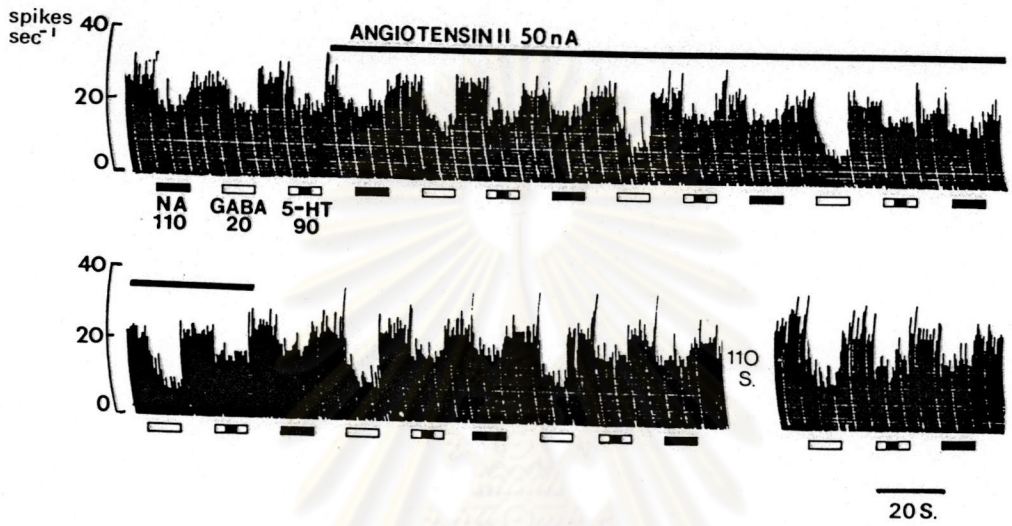

Figure 11. Effect of continuous application of angiotensin II (A II; 50 nA) on depressant action of nor-adrenaline (NA; 110 nA), ɣ-aminobutyric acid (GABA; 20 nA) and 5-hydroxytryptamine (5-HT; 90 nA) on Purkinje cell. The rate meter shows that A II selectively potentiates inhibitory action of GABA while the depression of neuronal activity caused by NA and 5-HT remained unaffected.

response to the excitants was observed following A II application with current upto 100 nA over the periods of 10 min. Examples of these results is shown in Figure 12.

Effect of Angiotensin II Antagonist (Saralasin) on A II Induced Potentiation of GABA Action

In Purkinje neurones (n = 3) which were consistently depressed by pulsatile cationic iontophoretic administered GABA (5 - 25 nA; 10 sec duration, 10 - 30 regular intervals), continuous microiontophoretic application of A II (50 - 80 nA) produced enhancement of depressant action. When saralasin (10 - 20 nA) were superimposed on A II application, the observed enhancement were gradually antagonized. However, the enhancement reconstituted after termination of saralasin. Recovery from A II effect were also observed following cessation of A II application, example of these results is shown in Figure 13.

Effects on Iontophoretic Application of GABA-Antagonist Bicuculline

The convulsant alkaloid bicuculline, a presumptive GABA antagonist (Curtis, Duggan, Felix and Johnson, 1971) was selected as a tool to investigate the possibility of GABA involement in A II effects. The methochloride derivative of the alkaloid was chosen because it was more resistant to hydrolysis than the bicuculline base under physiological condition (Olsen, Ban, Miller and Johnston 1975).

Effect of bicuculline on A II action

On 5 neurones, pulsatile iontophoretic application (10 sec duration and at 10 sec regular intervals) of GABA (5 - 20 nA; n = 5),

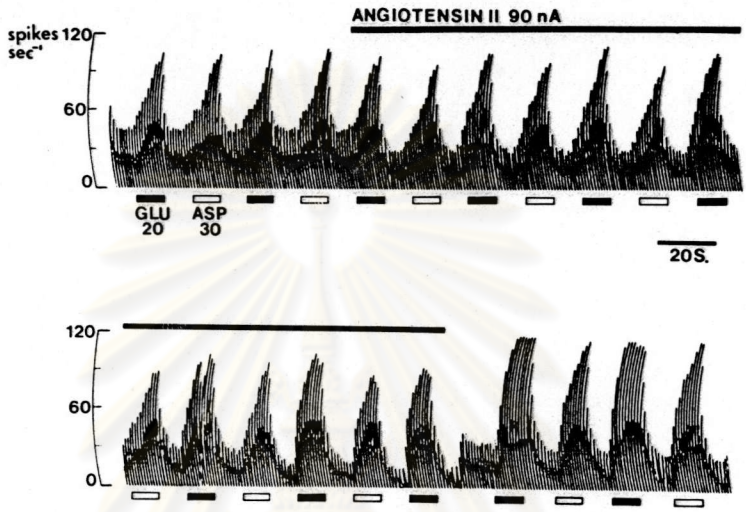

Figure 12. Effect of continuous application of angiotensin II (A II; 90 nA) on excitant action of glutamate (GLU; 20 nA) and aspartate (ASP; 30 nA) on Purkinje cell. The rate meter shows that no change in the neuronal responsiveness to the excitants was observed following A II application over the periods of 4 min, while A II produced approximately 50 percent depression of spontaneous discharge.

Figure 13. Effect of angiotensin II antagonist saralasin on A II induced potentiation of GABA action. This record shows that continuous application of A II (70 nA) produced enhancement of depression action of GABA (5 nA) on Purkinje cell discharge. When saralasin (20 nA) was superimposed on A II application, the observed enhancement was gradually antagonize. However, the enhancement was reconstitued after termination of saralasin. Recovery to the control level of GABA produced depression was also observed following cessation of the A II application.

A II (50 - 80 nA; n = 5) and GLY (60 - 70 nA; n = 3) produced consistent depression of Purkinje cells tested. When continuous iontophoretic application of bicuculline methochloride (BMC; 20 - 30 nA, positive current) were superimposed on the depressant pulses, BMC antagonized the depression effect of GABA and A II by 70 - 100 % within 0.5 - 4 min. While the depression of neuronal activity caused by the control agonists GLY (n = 3) remained unaffected. Example of these results is shown in Figure 14.

Effect of bicuculline on A II produced potentiation of GABA

In Purkinje neurones (n = 3) which were consistently depressed by pulsatile iontophoretic application (10 sec duration and 10 sec regular intervals) of GABA (5 - 20 nA, n = 3) GLY (10 nA, n = 1) and 5-HT (50 - 60 nA, n = 2), continuous iontophoretic applications of A II (50 - 70 nA) produced enhancement of GABA induced firing depression without having any effect on action of 5-HT and GLY. Following superposition of BMC iontophoresis, A II induced enhancement of GABA action were gradually antagonized. However, subsequent manifestation of the enhancement under persistant A II ejection were also observed after termination of BMC application. The recovery from A II effect were also observed following cessation of A II application. Example of these results is shown in Figure 15.

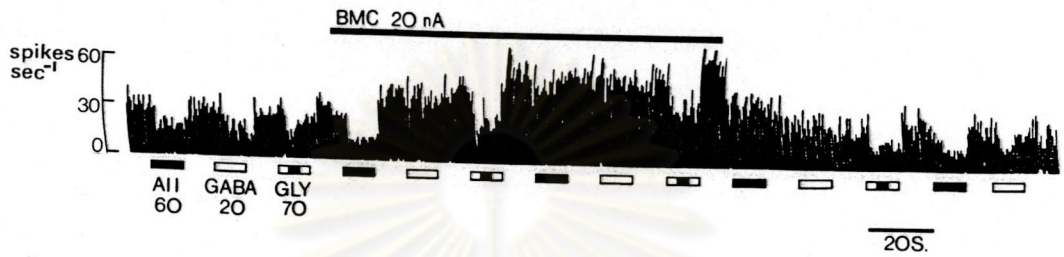

Figure 14. Effect of microiontophoretic administration of GABA antagonist bicuculline methochloride (BMC; 20 nA) on response of a Purkinje cell to iontophoretic application A II (60 nA), GABA (20 nA) and GLY (70 nA)。 The rate meter shows that 25 sec. after commencement of the application BMC selectively and reversibly antagonized the depressant effect of GABA and A II。 While the depression of neuronal activity caused by the control agonist GLY remained unaffected。

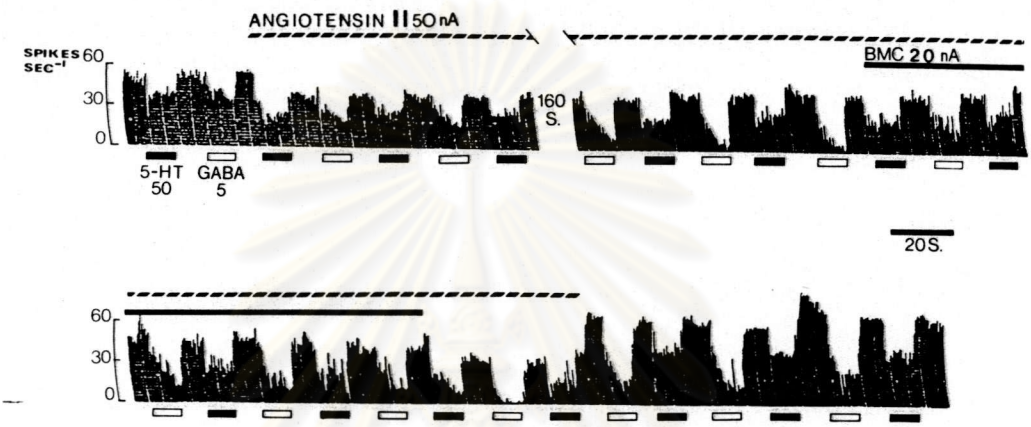

Figure 15. Effect of GABA antagonist (BMC) on A II induced potentiation of GABA action. The rate meter shows that continuous application of A II (50 nA) produced enhancement of depression action of GABA (5 nA) on Purkinje cell discharge, while the neuronal activity caused by 5-HT (50 nA) remained unaffected. When BMC (20 nA) was superimposed on A II application, the observed enhancement was gradually antagonize. However, the enhancement was reconstitued after termination of BMC. Recovery to control level of GABA produced depression was also observed following cessation of A II application.