Chapter III

Results

The effect of VPA and HPP on amino acid neurotransmitters in cerebral cortex of freely moving rats: an *in vivo* microdialysis study

The experiment was divided into 6 groups. Control groups (NSS-treated rats and PEG-400 treated rats), VPA-treated rats (220 mg/kg, and 440 mg/kg), HPP-treated rats (80 mg/kg and 160 mg/kg) (n=5 in each group).

The excitatory neurotransmitters in question are glutamate and aspartate whereas GABA and glycine are the ones with inhibitory effect. Alteration of amino acid neurotransmitter's levels during 1 hour before and 3 hours after treatment was expressed as percentage of change from basal value which was determined from three consecutive samples before the administration of the test substances. Total changes of amino acid levels in dialysate from rat cerebral cortex in 6 groups of experiment expressed as mean of percentage of change from basal value $\pm$ standard error of mean (S.E.M.), were compared.

In control groups, the effect of PEG-400 on cortical GABA, glycine, glutamate and aspartate level was not statistically different from those of NSS (Figure 7,9,11 and 13). VPA in the dose of 220 mg/kg B.W. did not exert any significant effect on the total change of GABA, glycine, glutamate and aspartate levels (Figure 8,10,12 and 14). Nevertheless, in higher dose (400 mg/kg B.W.), VPA did reduce total change of glutamate but not aspartate level (Figure 12 and 14). No significant effect of high dose of VPA (440 mg/kg B.W.) was noted on the level of inhibitory amino acids, GABA and glycine (Fig 8 and 10).

HPP in the dose of 80 and 160 mg/kg B.W. significantly decreased the total change of glutamate level (Figure 12). A marked decrease in the level of glutamate was elicited exclusively by HPP in the dose dependent manner (Figure 11). On the

contrary, HPP did not exert any major effect on either total change of GABA or glycine or aspartate levels (Fig 8, 10 and 14).

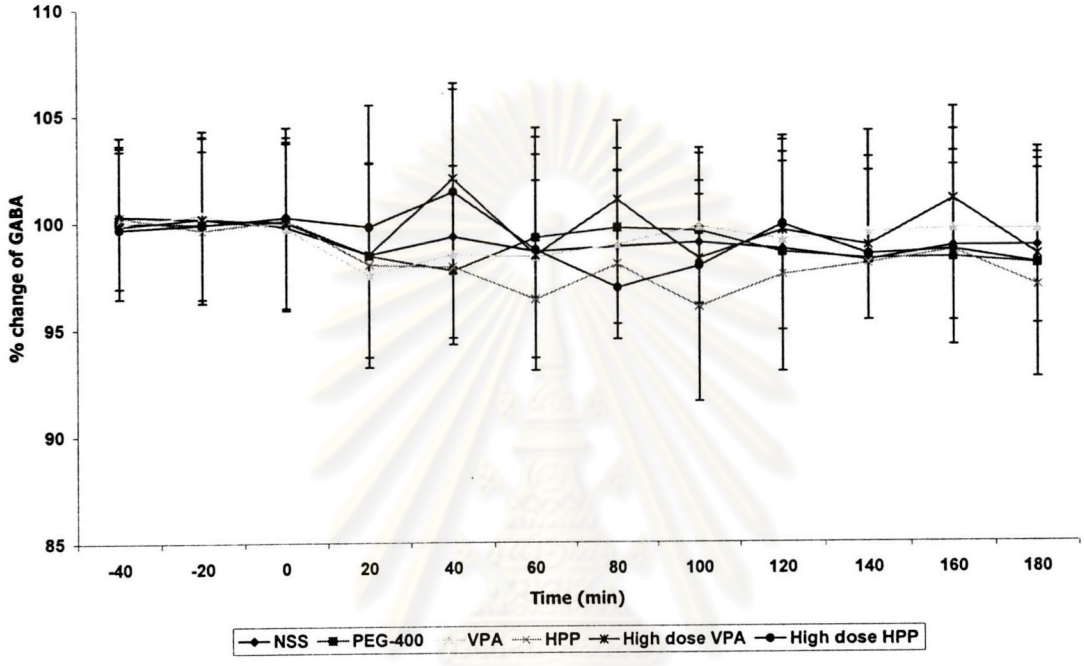


Figure 7 The level of GABA in rat cerebral cortex during 1 hour before and 3 hours after the administration of test substances. NSS, PEG-400, VPA (220 and 440mg/kg B.W.) and HPP (80 and 160 mg/kg B.W.) were intraperitoneally injected into different group of rat (n=5 in each group).

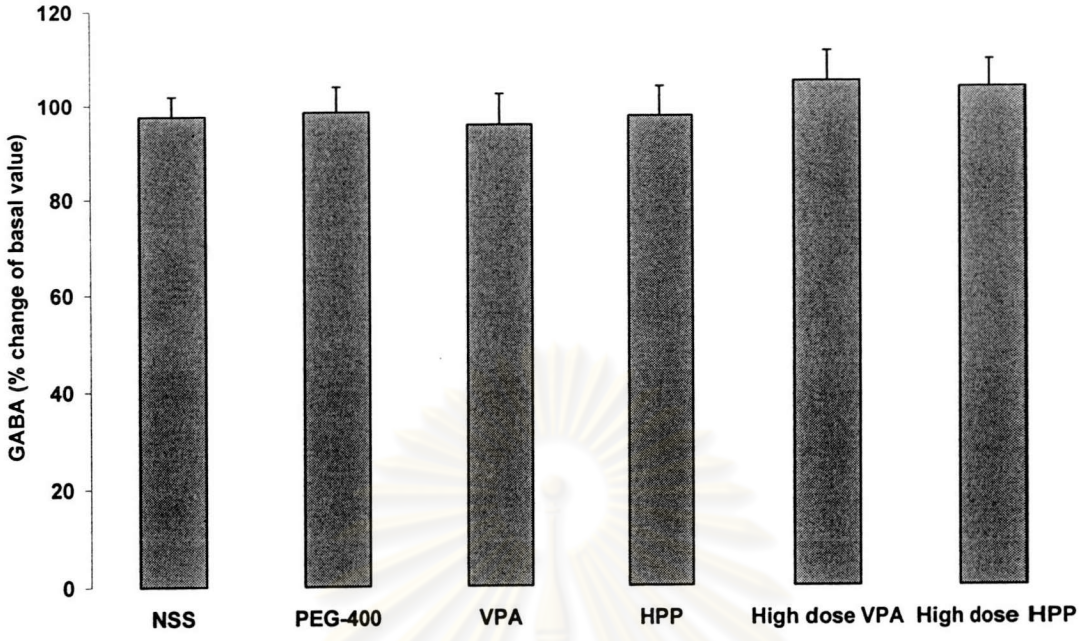


Figure 8 Total change of GABA in the dialysate from rat cerebral cortex comparing between 6 groups of experiment.

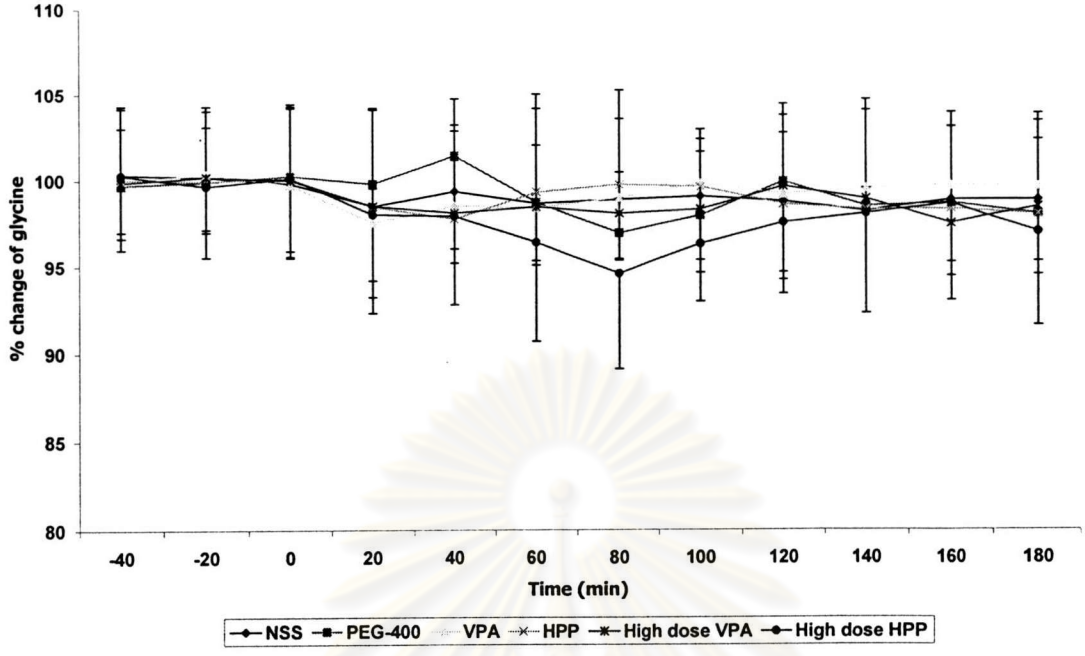


Figure 9 The level of glycine in rat cerebral cortex during 1 hour before and 3 hours after the administration of test substances. NSS, PEG-400, VPA (220 and 440mg/kg B.W.) and HPP (80 and 160 mg/kg B.W.) were intraperitoneally injected into different group of rat (n=5 in each group).

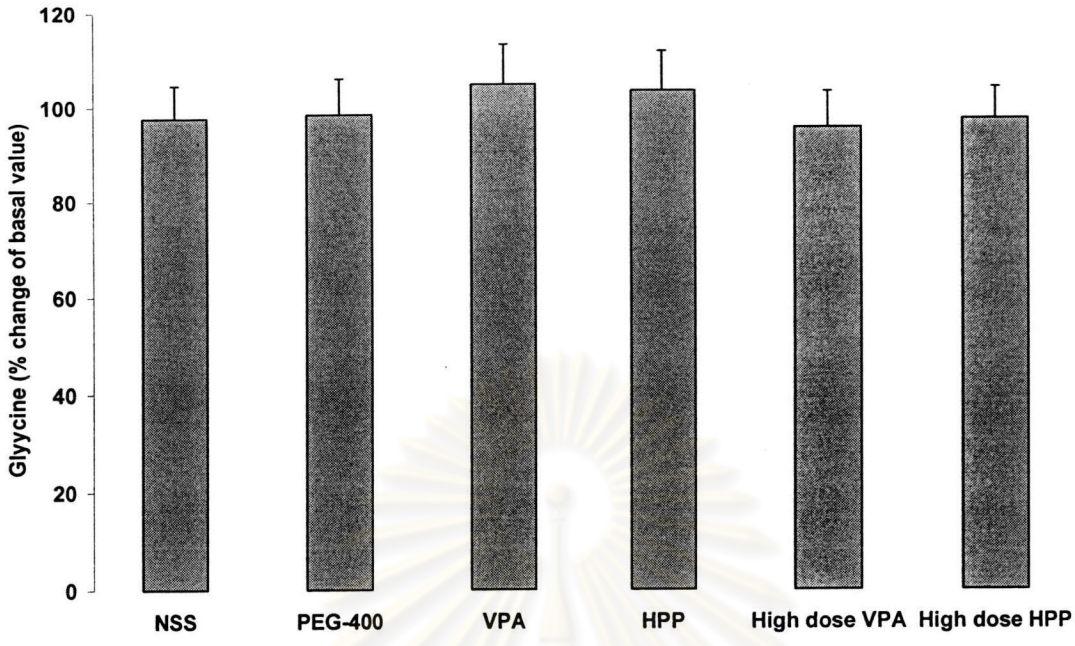
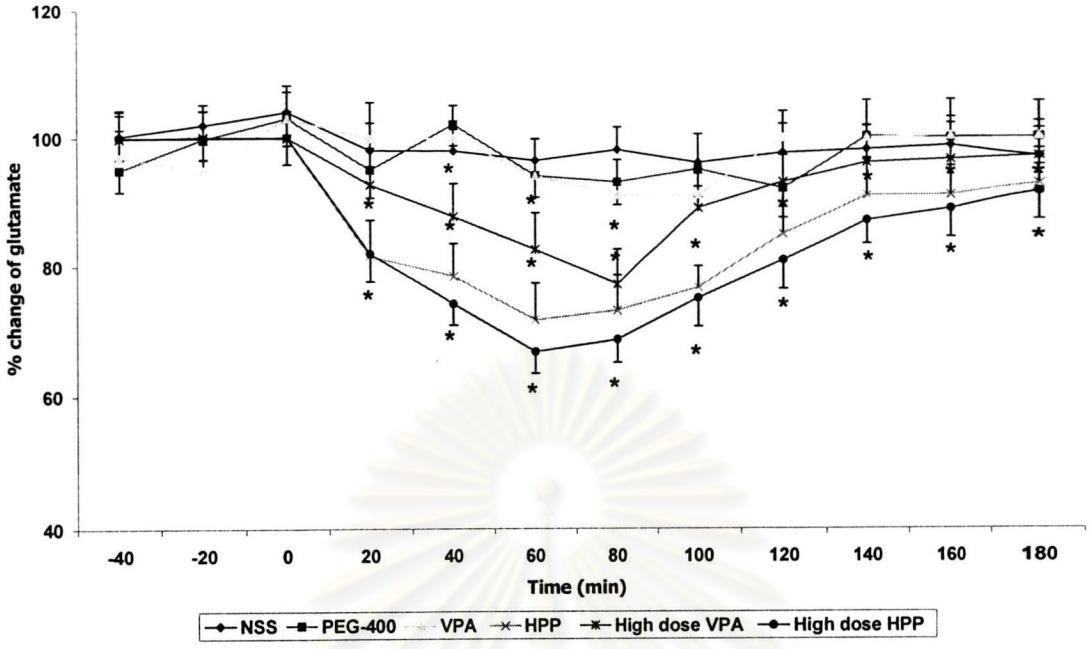
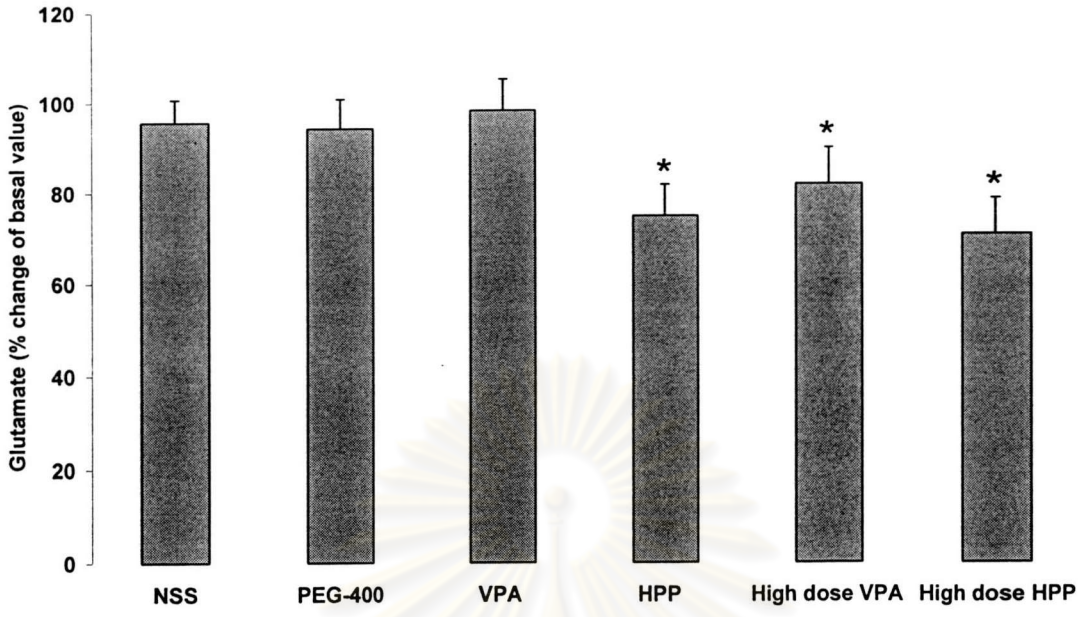


Figure 10 Total change of glycine in the dialysate from rat cerebral cortex comparing between 6 groups of experiment.



* $p < 0.05$ denotes statistically significant difference from PEG-400

Figure 11 The level of glutamate in rat cerebral cortex during 1 hour before and 3 hours after the administration of test substances. NSS, PEG-400, VPA (220 and 440mg/kg B.W.) and HPP (80 and 160 mg/kg B.W.) were intraperitoneally injected into different group of rat (n=5 in each group).



* $p < 0.05$ denotes statistically significant difference from PEG-400

Figure 12 Total change of glutamate in the dialysate from rat cerebral cortex comparing between 6 groups of experiment.

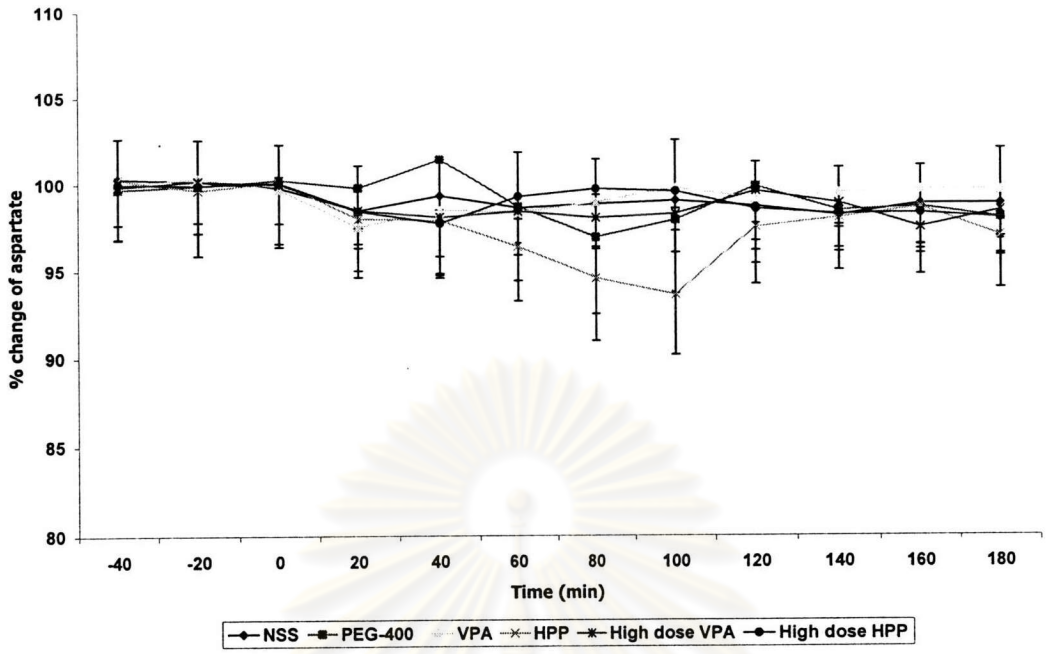


Figure 13 The level of aspartate in rat cerebral cortex during 1 hour before and 3 hours after the administration of test substances. NSS, PEG-400, VPA (220 and 440mg/kg B.W.) and HPP (80 and 160 mg/kg B.W.) were intraperitoneally injected into different group of rat (n=5 in each group).

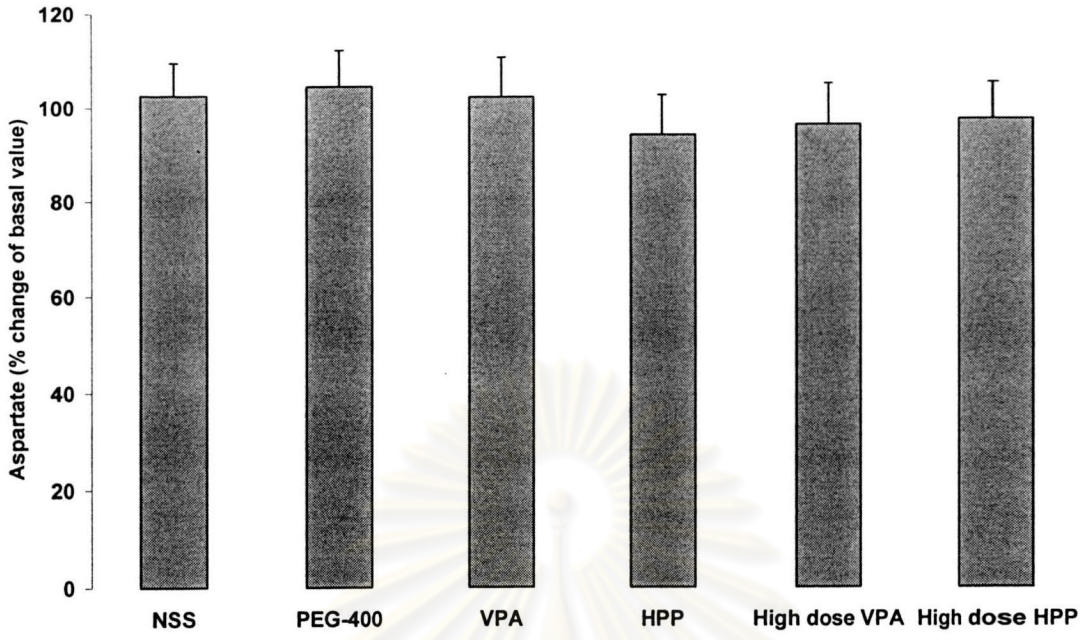


Figure 14 Total change of aspartate in the dialysate from rat cerebral cortex comparing between 6 groups of experiment.

The effect of HPP on $GABA_A$, Glycine and NMDA receptors in acutely dissociated rat hippocampal neurons

1. The viability and morphological appearance of acutely dissociated rat hippocampal neurons

After dissociation, some of hippocampal neurons were viable. Most of the dissociated hippocampal pyramidal neurons still had a typical morphological shape. Less than 10% of the neurons were still viable after plating into a recording chamber for 4 hrs. The photomicrograph of a representative hippocampal pyramidal neuron acutely dissociated from 21-day-old male Wistar rat has shown in figure 15 (Photo from Assist. Prof. Dr. Thongchai Sooksawate).

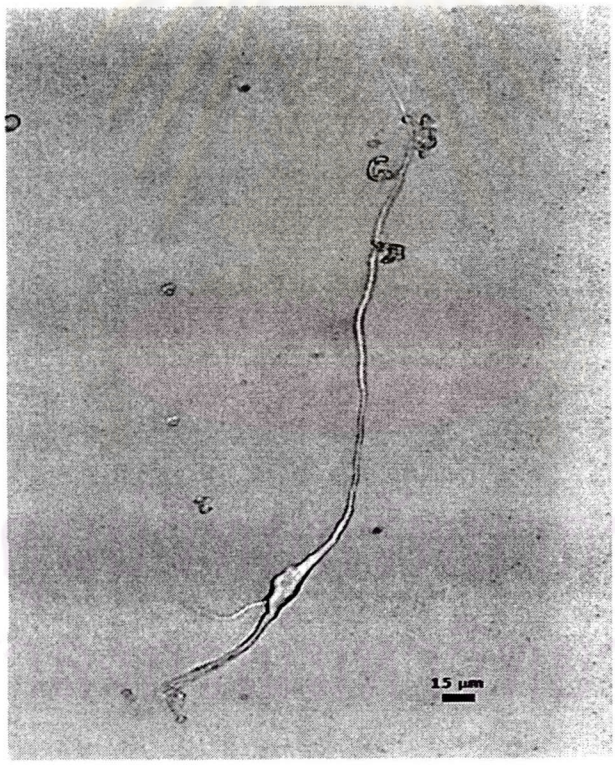


Figure 15 The photomicrograph of a representative hippocampal pyramidal neuron acutely dissociated from 21-day-old male Wistar rat by a method of Sooksawate and Simmonds (1998). Scale bar =15 µm.

2. The effect of HPP on $GABA_A$ receptor

2.1 Neuronal response to GABA

The inward currents evoked by a rapid application of increasing concentrations of 0.3-1,000 μM GABA demonstrated dose-dependent manner. The desensitization characteristics of the current were observed at high GABA concentration (10 μM GABA). The GABA concentration producing a maximal current amplitude was about 300 μM (Figure 16).

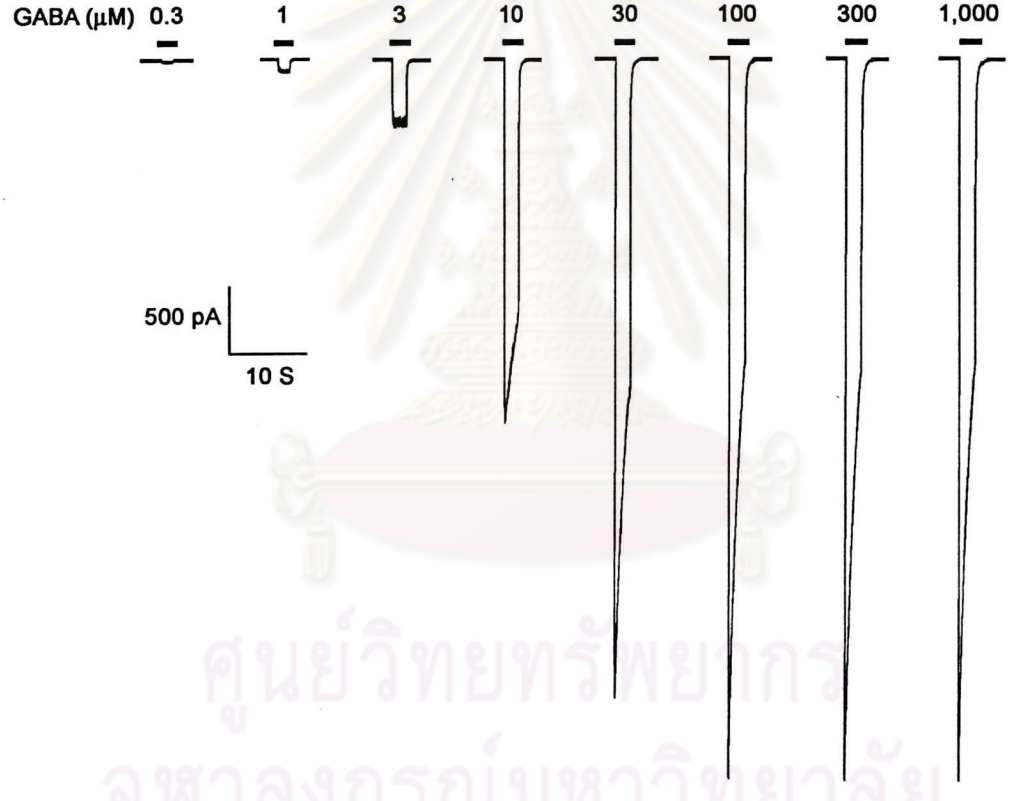


Figure 16 Representative current traces demonstrating whole-cell $GABA_A$ currents induced by increasing concentrations of 0.3-1,000 μM GABA to an acutely dissociated hippocampal pyramidal neuron from male Wistar rat aged 21 days. Drug applications were separated by at least 1-2 min interval and the duration was indicated by the solid line above the current traces. All record current traces are from the same neuron. Holding potential was –20 mV.

The EC_{50} values and Hill coefficient of the log concentration-response relationship obtained from the control neurons were 8.06 ± 0.14 μM and 1.63 ± 0.04 respectively (n=7) (Figure 17).

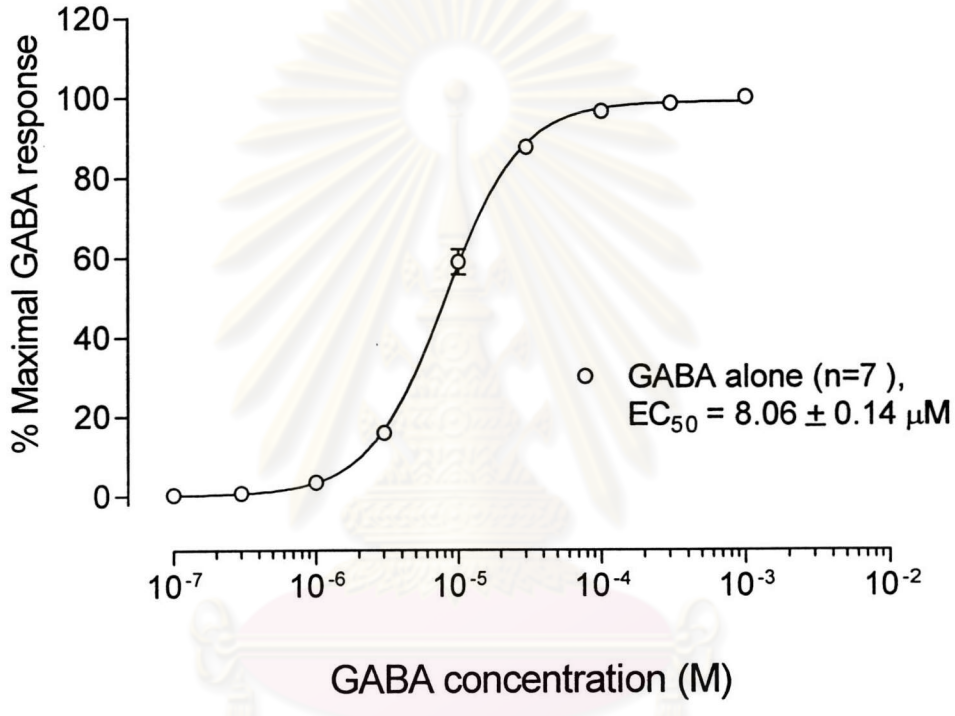


Figure 17 GABA log concentration-response relationship in acutely dissociated rat hippocampal pyramidal neurons. Each point is the mean ± S.E.M. of the current response, expressed as percentage of the maximal response.

2.2 Effects of bicuculline methochloride (BMC) and picrotoxinin (PTX) on the GABA-induced inward currents.

The inward currents induced by GABA could also be blocked by 20 μM bicuculline methochloride (BMC, a competitive antagonist of GABA at the $GABA_A$ receptor) and the concentration of BMC that inhibited $GABA_A$ currents completely was 20 μM, (n=7) (Figure 18).

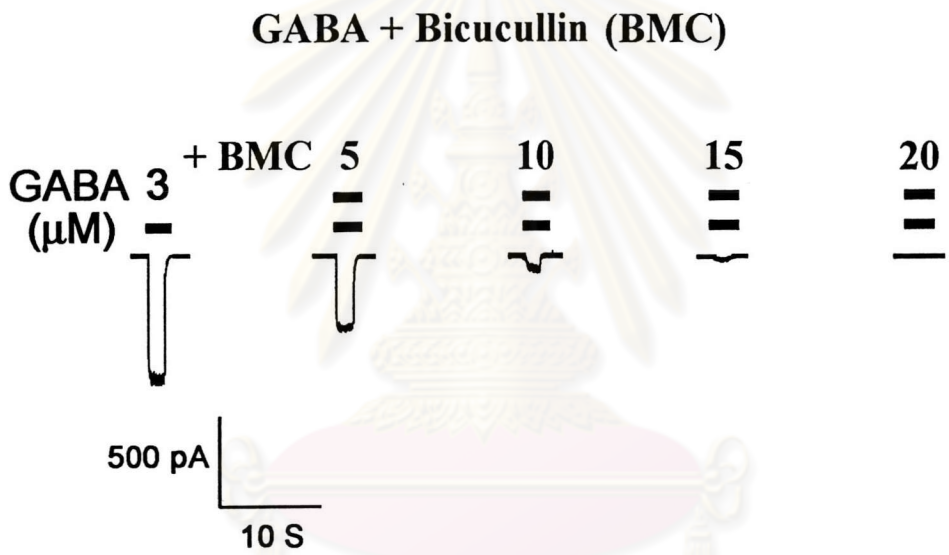


Figure 18 Representative current traces demonstrating the inhibition of the $GABA_A$ currents by 5, 10, 15 and 20 μM bicuculline methochloride (BMC).

The inward currents induced by GABA could also be blocked by 10 μM picrotoxinin (PTX, a noncompetitive antagonist of GABA at the $GABA_A$ receptor)and the concentration of PTX that inhibited $GABA_A$ currents completely was 200 μM, (n=7) (Figure 19).

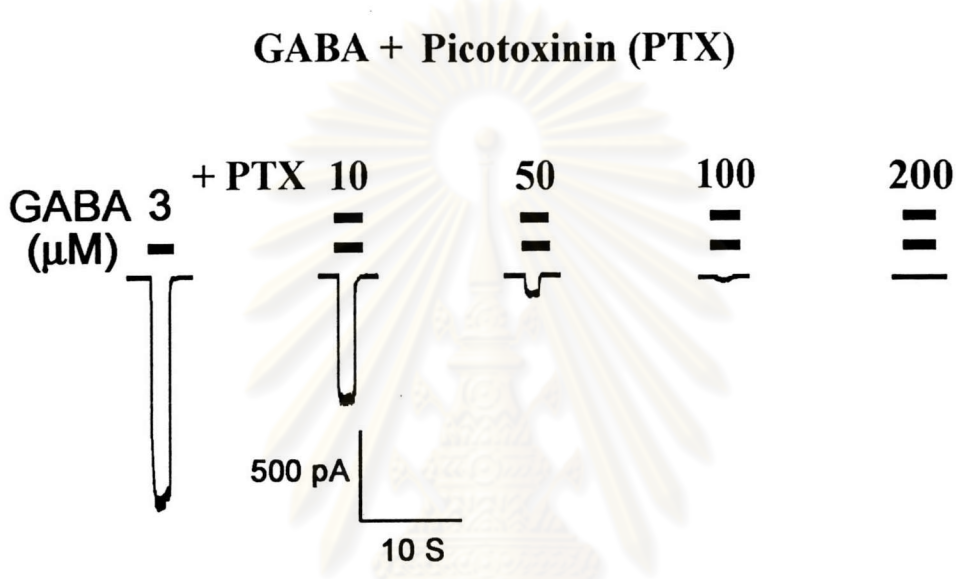


Figure 19 Representative current traces demonstrating the inhibition of the $GABA_A$ currents by 10,50,100 and 200 μM picrotoxinin (PTX).

The inhibition of the $GABA_A$ currents by BMC and PTX were significant difference (Dunnett's test, $P<0.001$ n=7) compared with 3 μM of GABA are shown in figure 20.

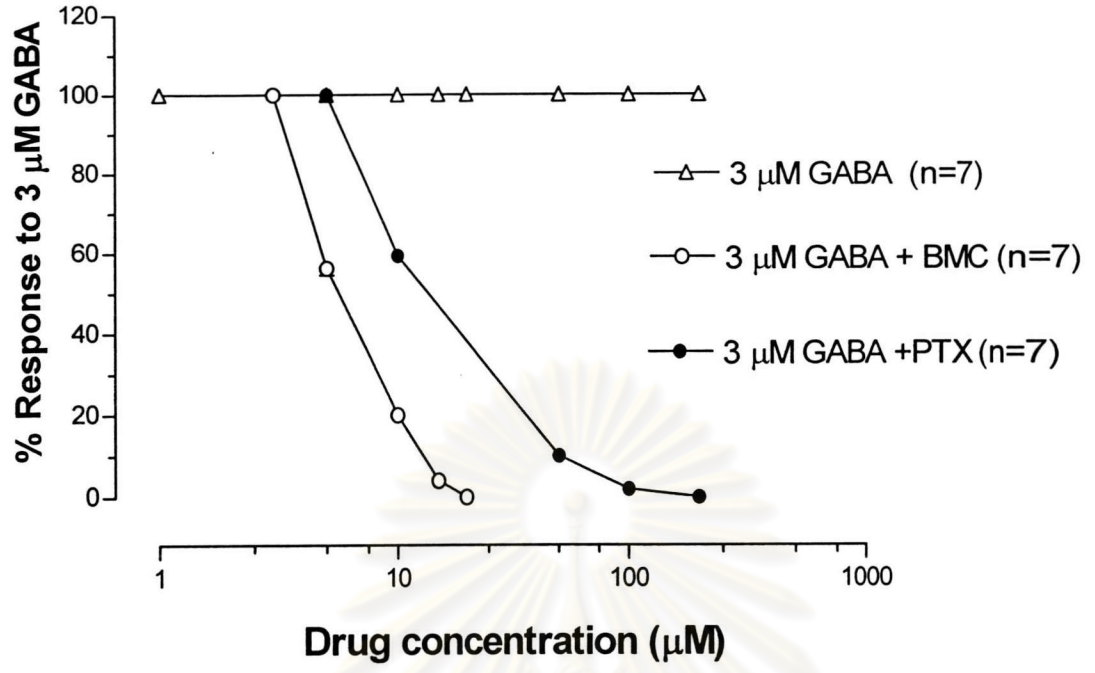


Figure 20 The concentration-dependent inhibition of the $GABA_A$ currents by bicuculline methochloride (BMC) and picrotoxinin (PTX).

2.3 Effect of diazepam (DZP) on the GABA-induced inward currents.

Diazepam, concentration up to 1,000 μM, did not induce inward current in the absence of GABA (n=7) (Figure 21 A). Diazepam, concentrations from 0.001-10 μM, produced the potentiation of the $GABA_A$ currents in the concentration-dependent manner with maximal potentiation at 1 μM (n=7) (Figure 21 B).

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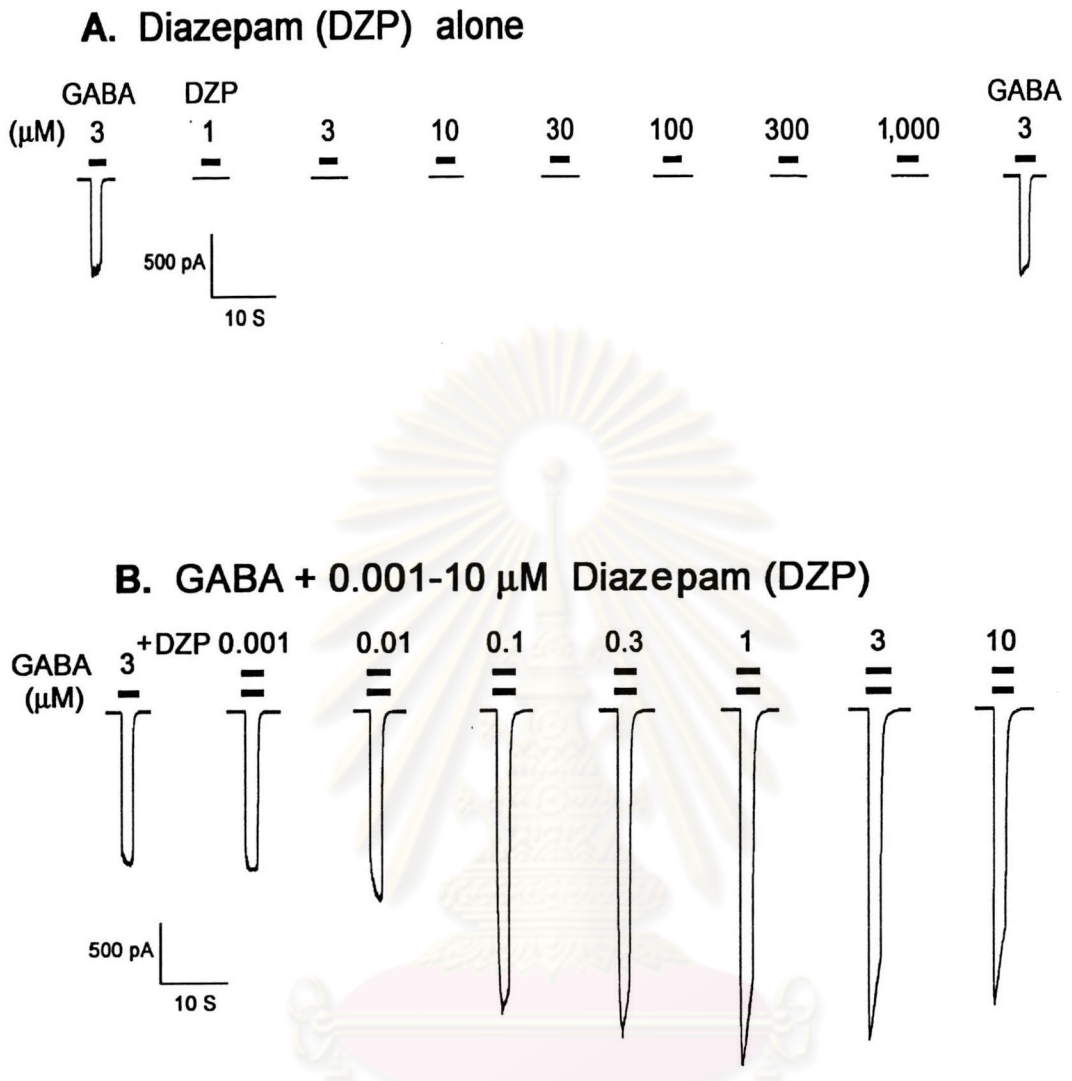

Figure 21 Representative current traces demonstrating the direct effect and potentiation of diazepam (DZP) on the $GABA_A$ currents. (A): DZP, up to 1,000 μM, did not induce inward current in the absence of GABA. (B): Co-application of 0.001-10 μM diazepam in the presence of 3 μM GABA produced the concentration-dependent manner. Note that the maximal potentiating effect of the $GABA_A$ currents by DZP exhibited at 1 μM DZP.

2.3 Effect of N-Hydroxymethyl-2-propylpentamide (HPP) on the inward current-induced by GABA

The inward currents induced by increasing concentrations of 0.1-300 μM HPP in the presence of 3 μM GABA are shown in figure 22, did not affect the GABA-induced inward currents (n=7).

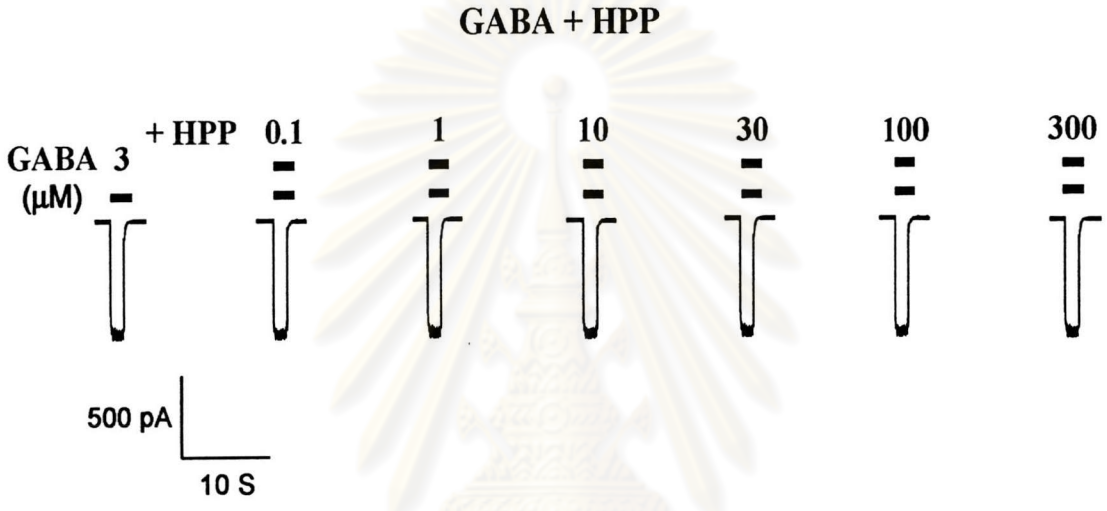

Figure 22 Representative current traces demonstrating that 0.1-300 μM HPP did not affect the GABA currents induced by 3 μM GABA.

The concentration-dependent of the $GABA_A$ currents by HPP was not significant difference (Dunnett's test, $P>0.05$ n=7) compared with 3 μM of GABA and the potentiation of the $GABA_A$ currents by DZP was significant difference (Dunnett's test, $P<0.001$ n=7) compared with 3 μM of GABA are shown in figure 23.

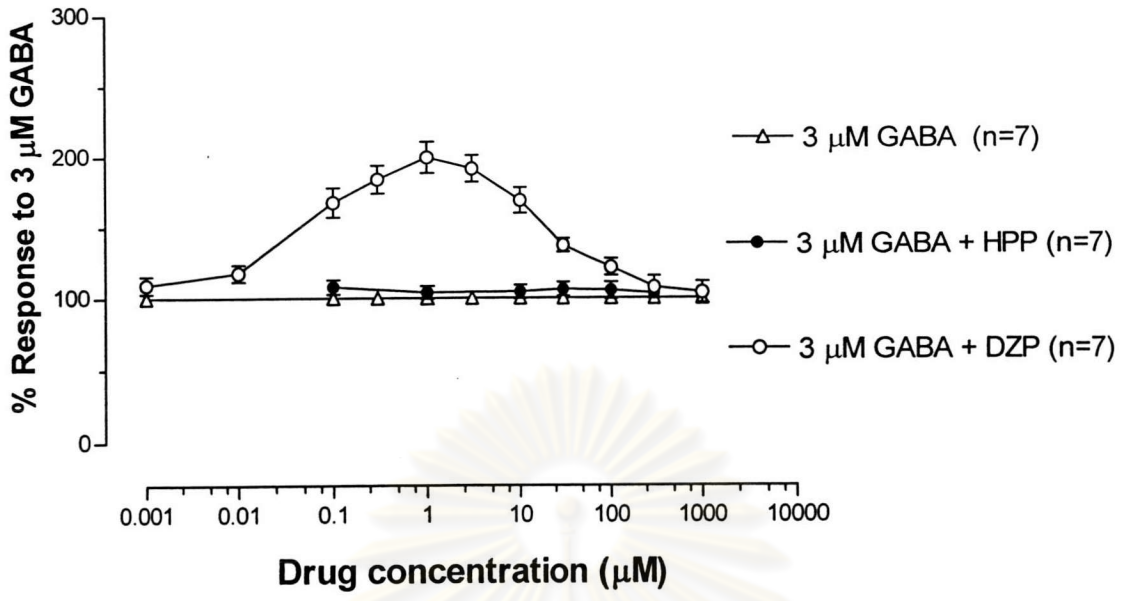

Figure 23 The concentration-dependent of the $GABA_A$ currents by HPP and potentiation of the $GABA_A$ currents by diazepam (DZP).

3. The effect of HPP on glycine receptor

3.1 Neuronal response to glycine

The inward currents evoked by a rapid application of increasing concentrations of 1-3,000 μM glycine demonstrated dose-dependent manner. The glycine concentration producing maximal current amplitude was about 1,000 μM (Figure 24).

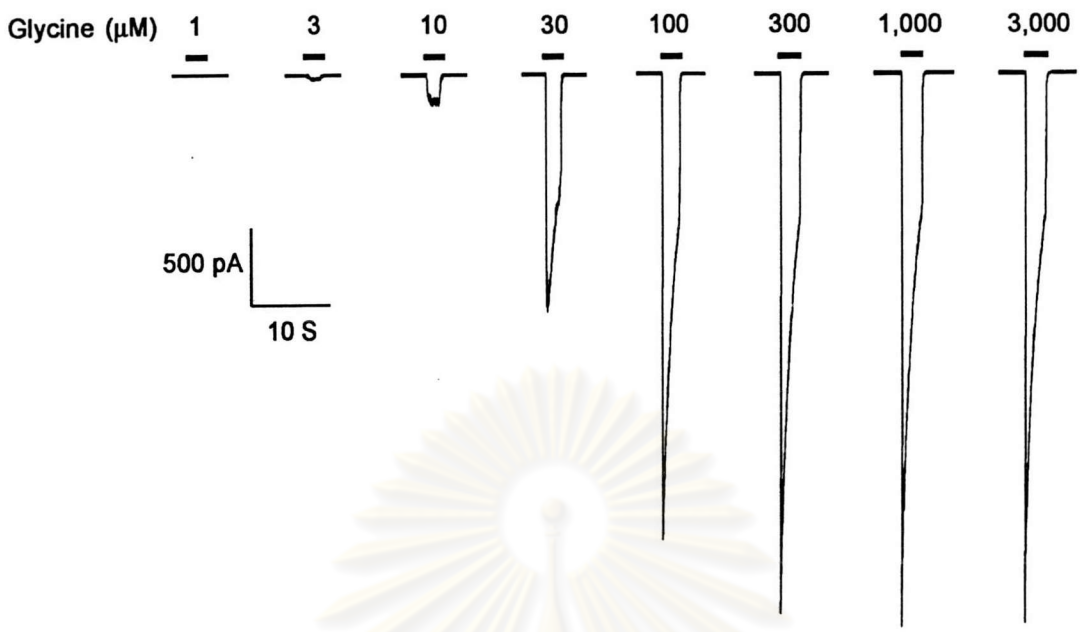

Figure 24 Representative current traces demonstrating whole-cell glycine currents induced by increasing concentrations of 1-3,000 μM glycine to an acutely dissociated hippocampal pyramidal neuron from male Wistar rat aged 21 days. Drug applications were separated by at least 1-2 min interval and the duration was indicated by the solid line above the current traces. All record current traces are from the same neuron. Holding potential (V_H) was –20 mV.

The EC_{50} values and Hill coefficient of the log concentration-response relationship obtained from the control neurons were 30.24 ± 0.25 μM and 1.6 ± 0.15 respectively (n=7) (Figure 25).

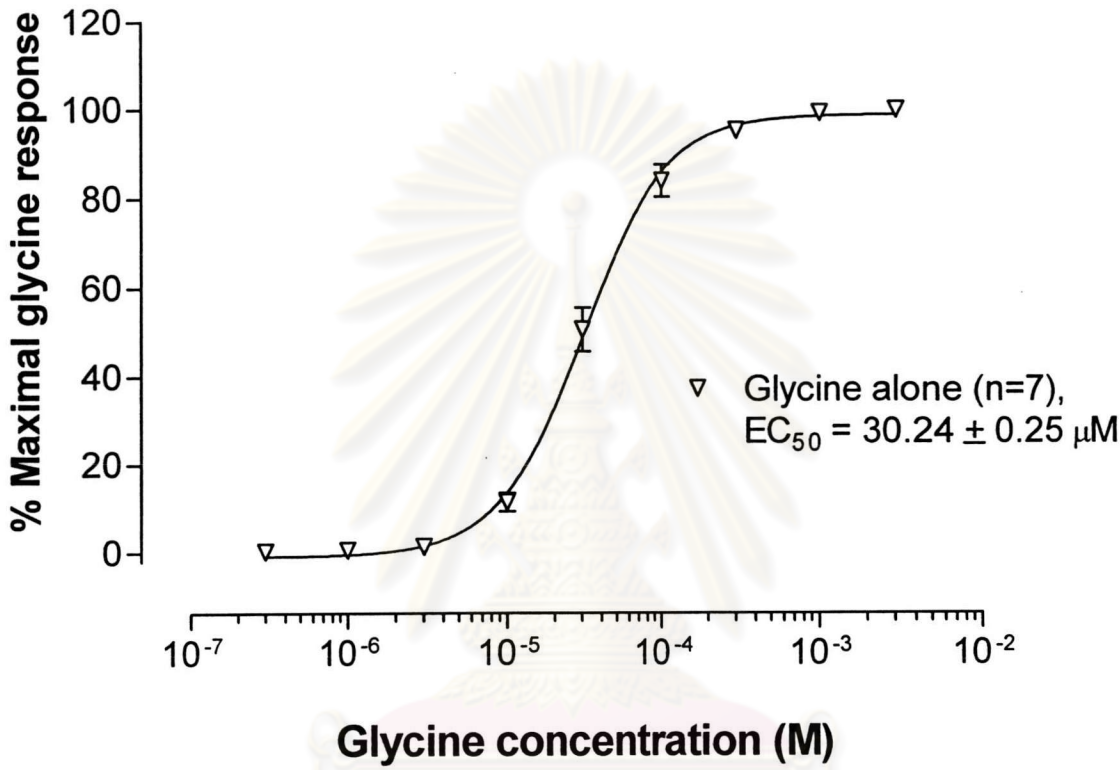

Figure 25 Glycine log concentration-response relationship in acutely dissociated rat hippocampal pyramidal neurons. Each point is the mean ± S.E.M. of the current response, expressed as percentage of the maximal response.

3.2 Effects of strychnine sulfate (STR) on the glycine-induced inward currents

The inward currents induced by glycine could be antagonized by 1 μM strychnine sulfate (STR, a competitive antagonist of glycine at the glycine receptor) and the concentration of STR that inhibited glycine currents completely was 20 μM, (n=7) (Figure 26).

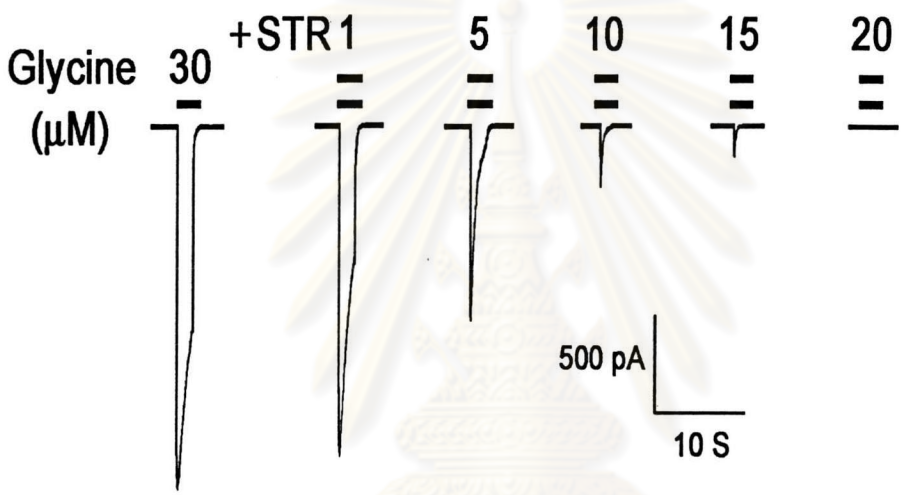

Figure 26 Representative current traces demonstrating inhibition of the glycine currents by 1,5,10,15 and 20 μM of strychnine sulfate (STR).

3.3 Effect of HPP on the glycine-induced inward currents

A concentration range of 0.1-300 μM HPP with 30 μM glycine, applied to a recorded neurons, did not affect the glycine-induced inward currents (n=7) (Figure 27).

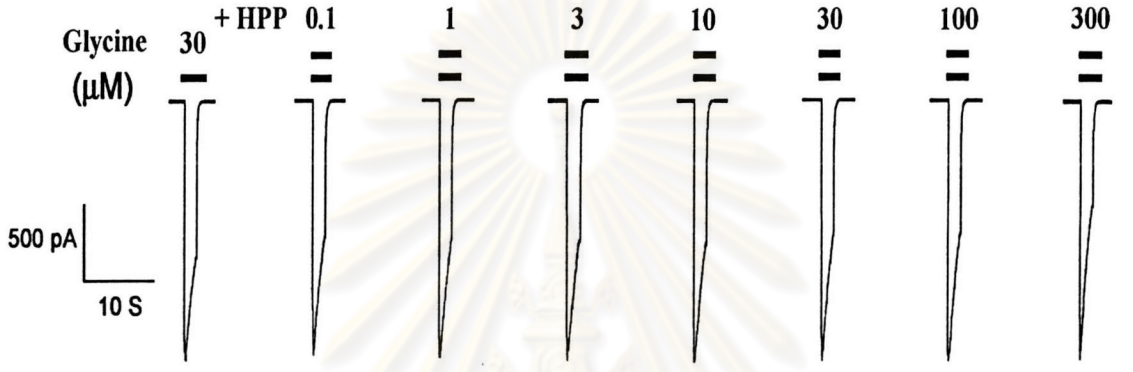

Figure 27 Representative current traces demonstrating that 0.1-300 μM HPP did not affect the glycine currents induced by 30 μM glycine

The concentration-dependent of the glycine currents by HPP was not significant difference (Dunnett's test, $P>0.05$ n=7) compared with 30 μM of glycine and the inhibition of glycine currents by STR was significant difference (Dunnett's test, $P<0.001$ n=7) compared with 30 μM of glycine are shown in figure 28.

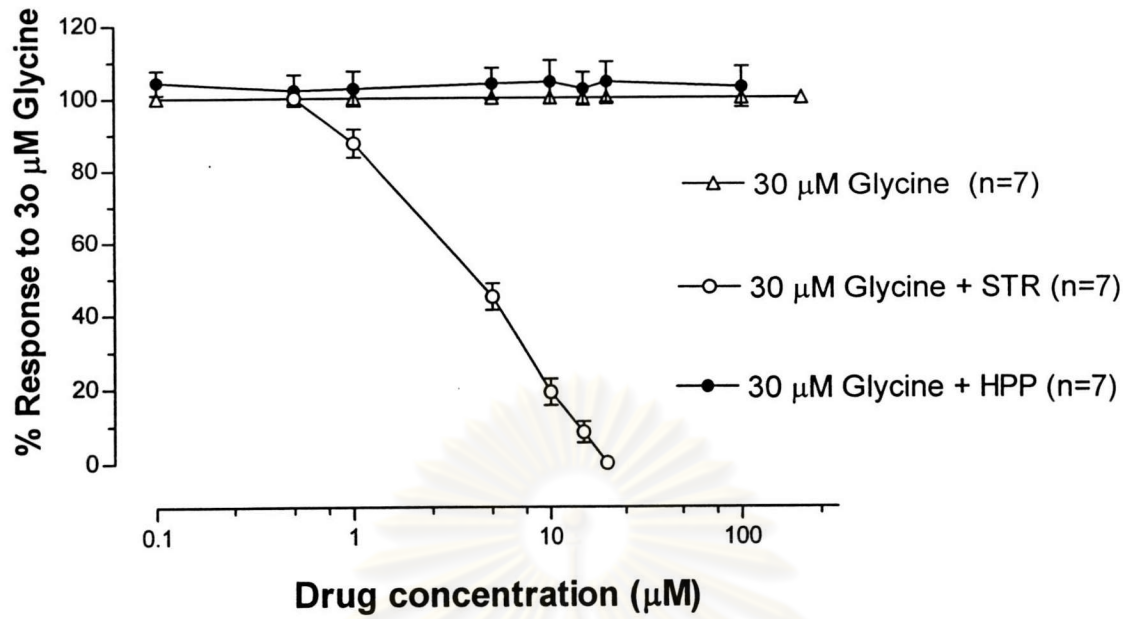

Figure 28 The concentration-dependent of the glycine currents by HPP and inhibition of the glycine currents by strychnine sulfate (STR).

4. The effect of HPP on NMDA receptor

4.1 Neuronal response to NMDA

The inward currents evoked by a rapid application of increasing concentrations of 1-800 μM NMDA demonstrated dose-dependent manner. The NMDA concentration producing a maximal current amplitude was about 500 μM (Figure 29).

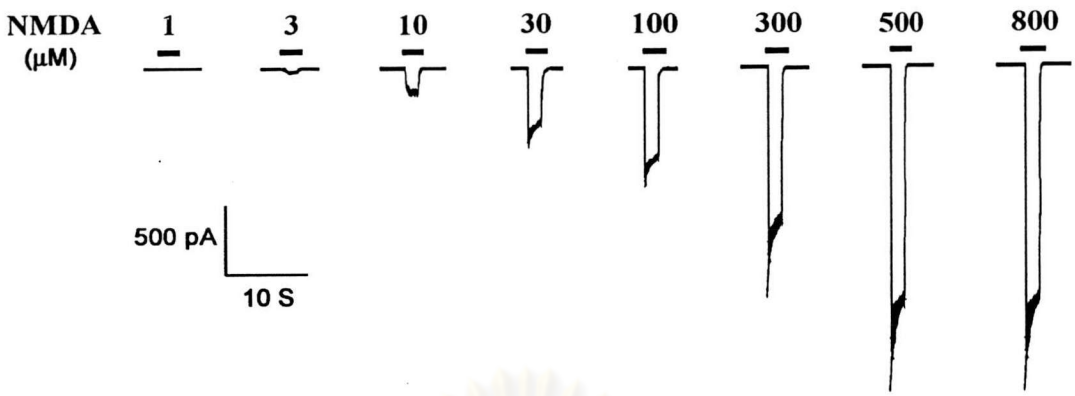

Figure 29 Representative current traces demonstrating whole-cell NMDA currents induced by increasing concentrations of 1-800 μM NMDA to an acutely dissociated hippocampal pyramidal neuron from male Wistar rat aged 21 days. Drug applications were separated by at least 1-2 min interval and the duration was indicated by the solid line above the current traces. All record current traces are from the same neuron. Holding potential (V_H) was –20 mV.

The EC_{50} values and Hill coefficient of the log concentration-response relationship obtained from the control neurons were 76.6 $\pm$ 0.24 μM and 0.91 $\pm$ 0.05 respectively (n=7) (Figure 30).

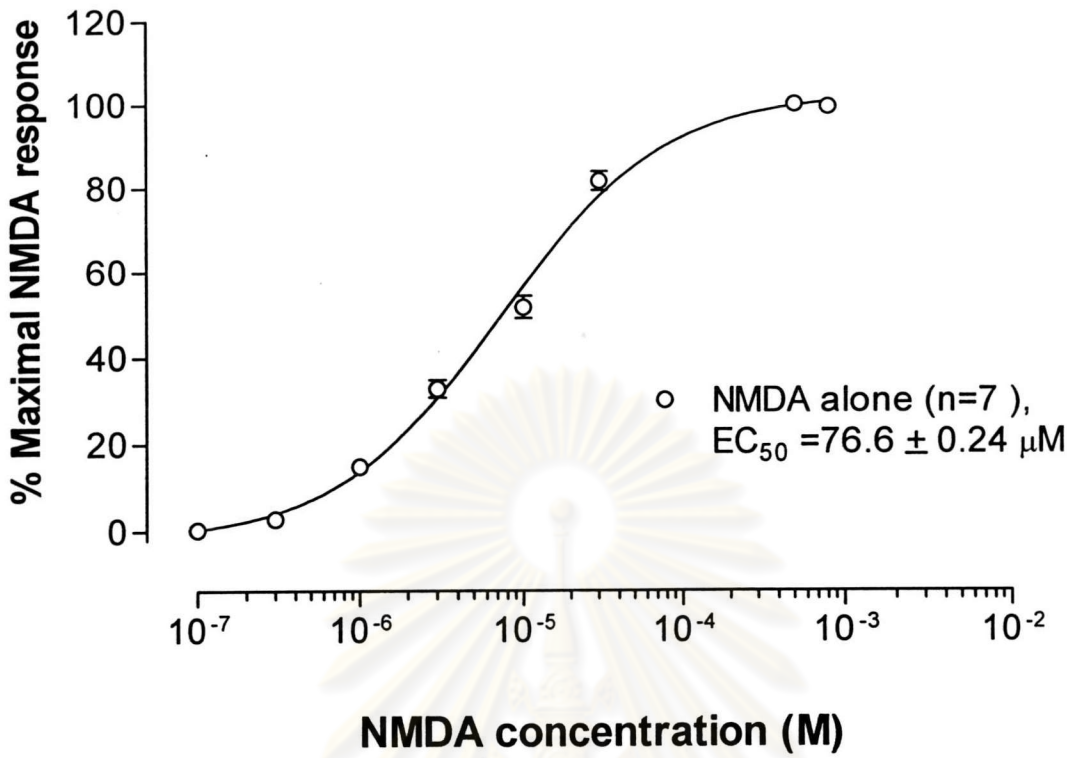

Figure 30 NMDA log concentration-response relationship in acutely dissociated rat hippocampal pyramidal neurons. Each point is the mean ± S.E.M. of the current response, expressed as percentage of the maximal response.

4.2 Effects of DL-2-Amino-5-phosphonopentanoic acid (AP-5) on the NMDA-induced inward currents

The inward currents induced by NMDA could be antagonized by 50 μM DL-2-Amino-5-phosphonopentanoic acid (AP-5, a selective antagonist for NMDA receptor) and the concentration of AP-5 that inhibited NMDA currents completely was 200 μM,(n=7) (Figure 31).

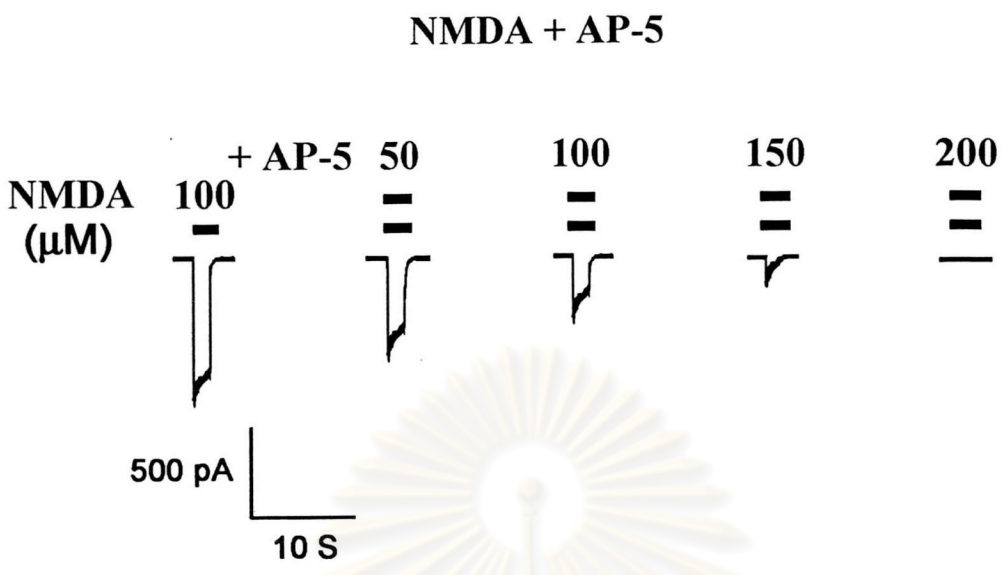

Figure 31 Representative current traces demonstrating inhibition of the NMDA currents by 50,100,150 and 200 of DL-2-Amino-5-phosphonopentanoic acid (AP-5).

4.3 Effect of HPP on the NMDA-induced inward currents

A concentration range of 0.1-300 μM HPP with 100 μM NMDA, applied to a recorded neurons, did not affect the NMDA-induced inward currents (n=7) (Figure 32).

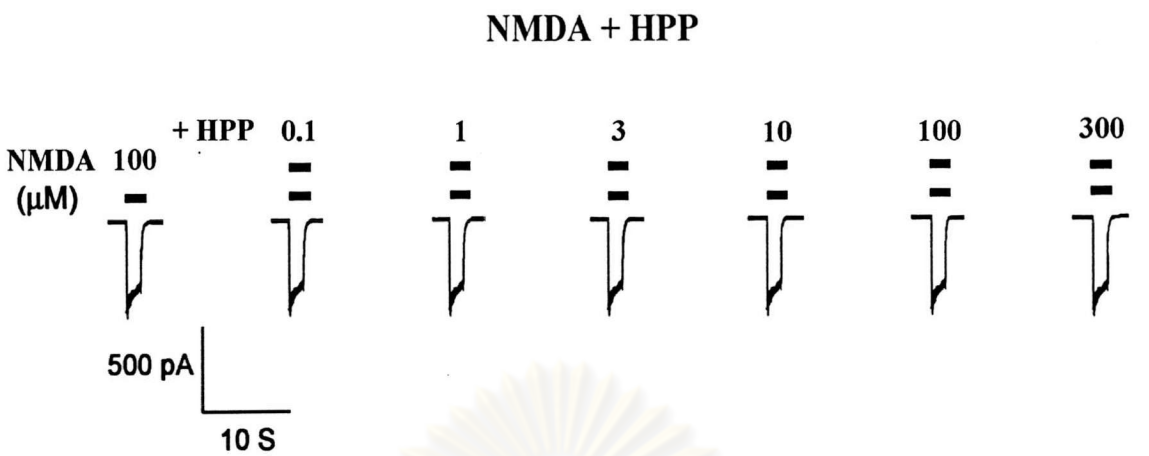

Figure 32 Representative current traces demonstrating that 0.1-300 μM HPP did not affect the NMDA currents induced by 100 μM NMDA.

The concentration-dependent of the NMDA currents by HPP was not significant difference (Dunnett's test, $P>0.05$ n=7) compared with 100 μM of NMDA and the inhibition of NMDA currents by AP-5 was significant difference (Dunnett's test, $P<0.001$ n=7) compared with 100 μM of NMDA are shown in figure 33.

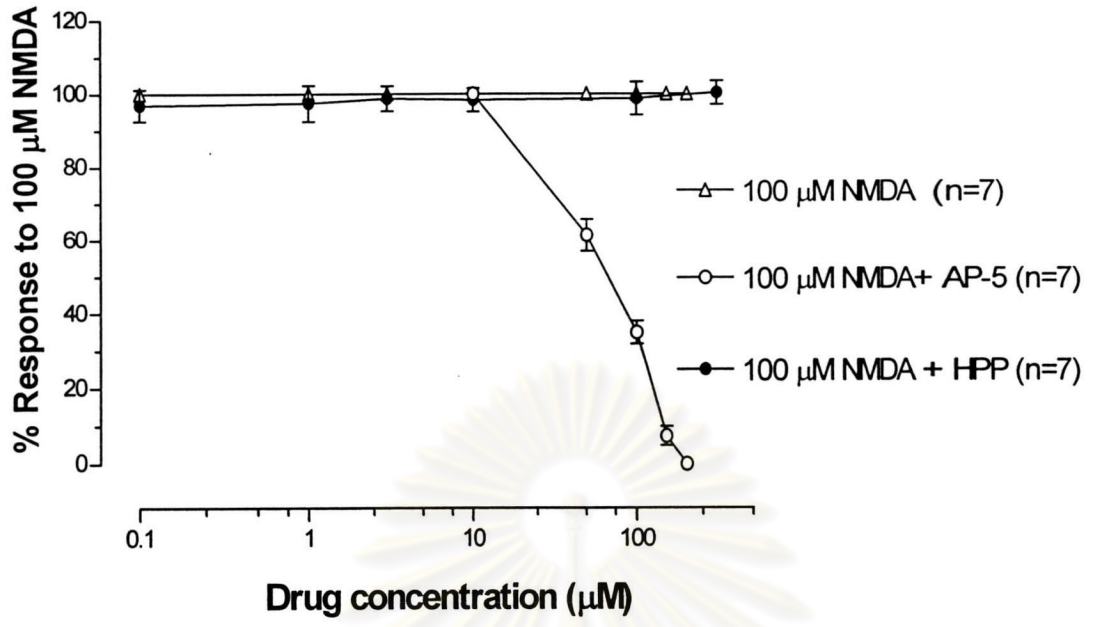

Figure 33 The concentration-dependent of the NMDA currents by HPP and the inhibition of NMDA currents by AP-5.