CHAPTER III

RESULTS

1. Characterization of glutamate-induced currents on oocytes injected with NR1A/NR2B mRNAs.

Bath application of glutamate and glycine to oocytes injected with NR1A/NR2B mRNAs elicited an inward current at clamp potential of –70 mV (figure 3.1). Calculation from the glutamate concentration response curve (figure 3.2) generated a Hill coefficient of 0.98 ± 0.12 (n = 4-6) and EC_{50} concentration of 2.26 ± 0.31 μM of glutamate(table 3.1).

The competitive inhibition of AP5, a known potent and selective competitive NMDA receptor antagonist, was also demonstrated by co-application of glutamate and its coagonist glycine (10 μM) in the presence of 10 μM AP5. The glutamate concentration response curve showed a shift to the right giving an average EC_{50} at 5.95 ± 0.37 μM in the presence of AP5 (n = 4-5, Figure 3.3). AP5 did neither change the maximal response nor the slope coefficient (+ 10 μM AP5: 1.40 ± 0.10) in comparison to to control conditions.

2. Effects of VPU and VPA on dose-response curves of glutamate-induced currents

Bath applications of 1-300 μM VPU demonstrated no direct or indirect effects of VPU on membrane currents of oocytes injected with NR1A/NR2B mRNAs.

The effects of VPU on glutamate-induced currents recorded from oocytes was also examined. Bath application of glutamate (0.01 – 300 μM) and 10 μM glycine induced inward currents proportional to concentration

given. Coapplication of VPU (10, 100 or 300 μM) resulted in a reversible inhibition of the response to glutamate. VPU reduced the glutamate-induced current competitively. Increment of VPU produced of the EC_{50} of glutamate was shown in table 3.1furthermore no significant effect on the Hill coefficient or maximal response to glutamate(Figure 3.4, 3.5, 3.6 and 3.7).

The effect of VPA on NMDA receptors were also investigated in *Xenopus* oocytes. In contrast to effect of VPU, it was found that 1 mM VPA did not significantly change the EC_{50}, maximal response and the slope coefficient exhibited by activation of NMDA receptor by glutamate (figure 3.8).

3. Effects of various concentration of VPU and AP5 on glutamate-induced currents

The inhibitory effects of VPU (0.1 – 300 μM) on glutamate-induced current which were recorded from oocytes injected with NR1A/NR2B mRNAs, was compared to the inhibitory effects of AP5 (0.1 – 300 μM), a known competitive NMDA receptor antagonist. The concentration of glutamate and glycine was fixed at 3 μM, a concentration producing an approximately half maximal response, and 10 μM, respectively. VPU and AP5 could inhibit the glutamate-induced currents as shown in figure 3.9. IC_{50} from inhibition curve were 23.55 ± 5.33 μM for AP5 (n = 3-5). VPU was less potent in blocking response, and the IC_{50} concentration of VPU could not be calculated.

4. Inhibitory effects of VPU in stepping holding potential

Investigation of the current-voltage (I-V) relationship (figure 3.10) was used to assess whether the inhibition induced by VPU was voltage-dependent or not. The I-V curve of 3 μM glutamate-induced currents recorded from

oocytes injected with NR1A/NR2B mRNAs were generated by stepping the holding potential from –150 to +50 mV by a step-increase of 10 mV. The lack of J-shaped current-voltage relation in the presence of VPU, clearly suggested that the inhibitory effects of VPU was voltage independent.

5. Effects of VPU on glutamate-induced current in the presence of various concentration of glycine

To investigate whether VPU blocks NMDA receptor currents by either competing or modulating at glycine site, glycine concentration-response curves (figure 3.11) were constructed by applying increasing concentrations of glycine with fixed 3 μM glutamate in the absence and presence of a fix concentration (100 μM) of VPU (figure 3.12). There was no significant changes in the maximal response and slope coefficient between those elicited in the presence or absence of VPU.

6. Effects of VPU on the stimulatory modulation of spermine

To address whether VPU directly or indirectly regulates spermine binding (at least from a functional level), the effect of spermine on NMDA receptor currents was examined in the absence and presence of VPU. The graph in figure 3.13 demonstrates that VPU produced a significant reduction in the potentiation of NMDA receptor responses by spermine ($P < 0.001$).

To examine the effects of spermine on VPU inhibition, VPU was applied in various concentrations to create the inhibition curve in the presence and absence of spermine (figure 3.14). Only high concentrations (30, 100 and 300 μM) of VPU could counteract the stimulatory effects of spermine.

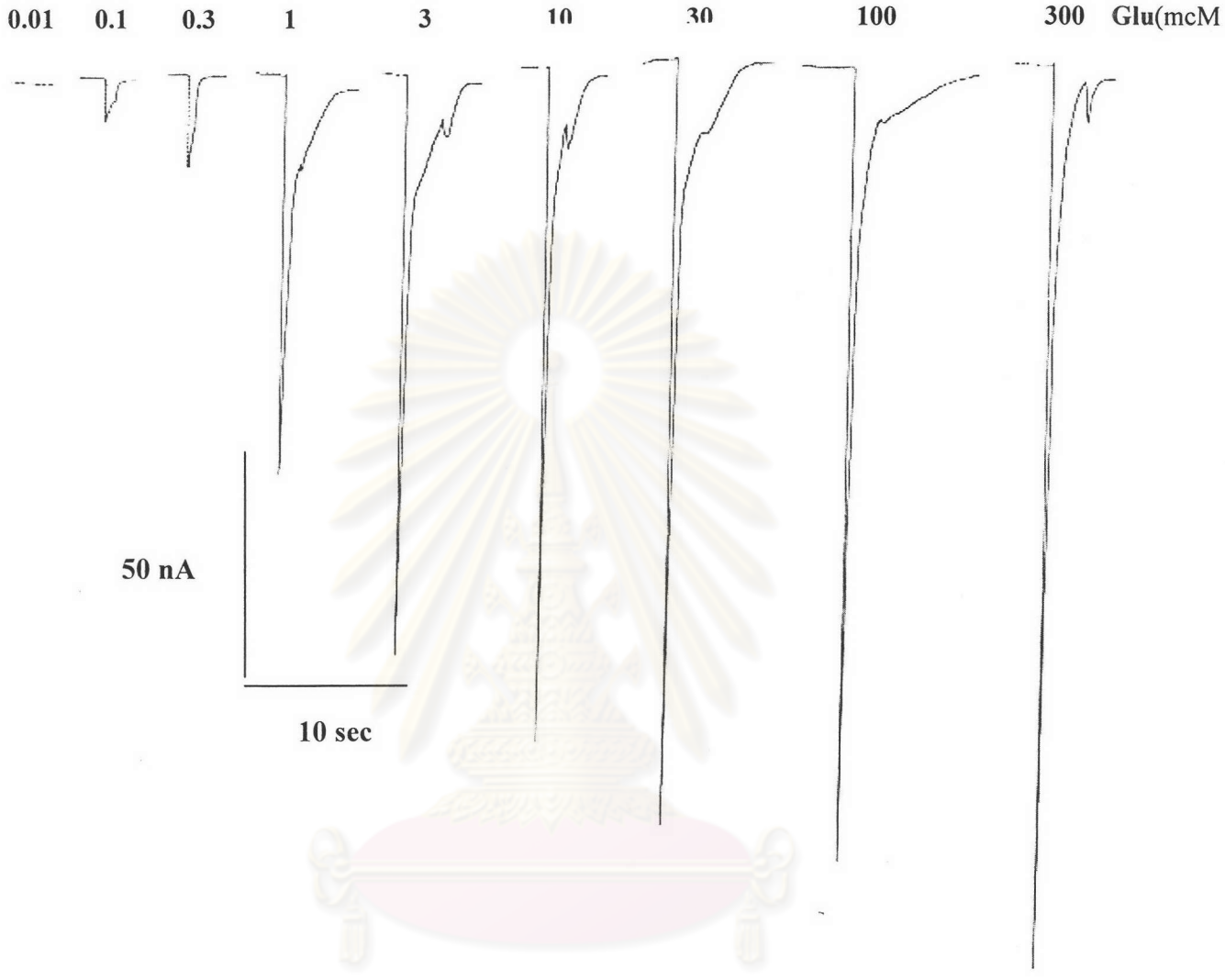

Figure 3.1 Illustration of currents evoked by 0.01,0.1, 0.3, 1, 3, 10, 30, 100 and 300 μ M glutamate plus 10 μM glycine on NR1A/NR2B receptors expressed in *Xenopus* oocytes injected with mRNA (holding potential: V_h = -70 mV).

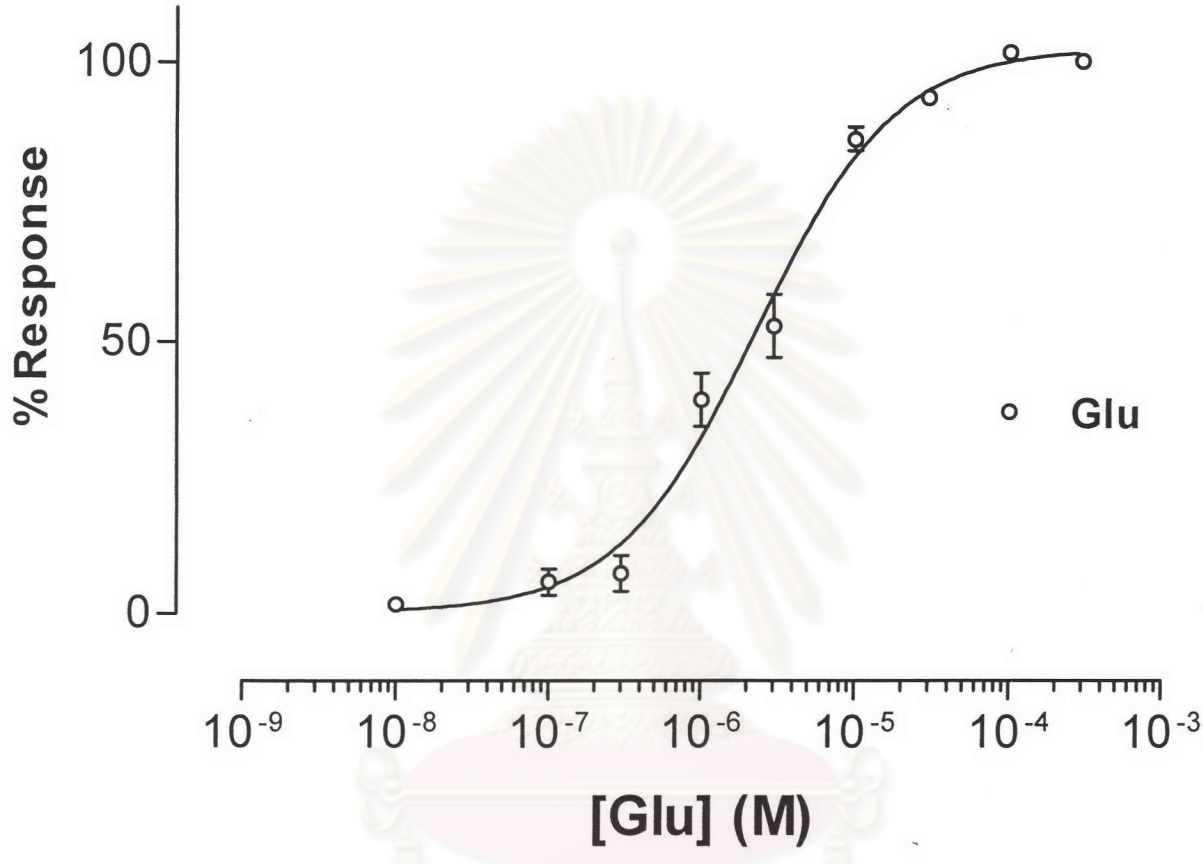

Figure 3.2 Glutamate log concentration-response relationship. Each point is the mean ± S.E.M. of the current response of 4-6 oocytes, expressed as percentage of the maximal response.

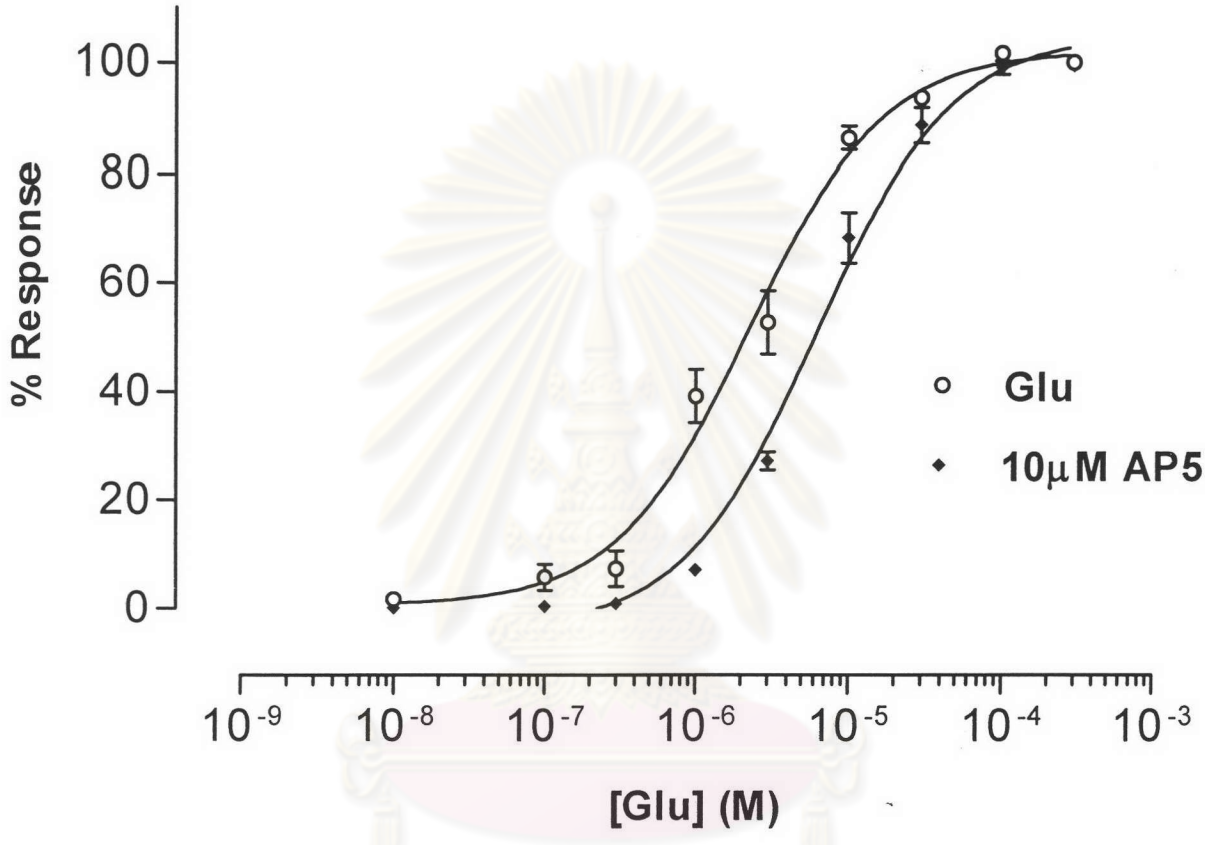

Figure 3.3 Glutamate log concentration responses curves demonstrating the inhibitory effect of 10 μM AP5. AP5 shifted the glutamate concentration-response curve to the right without changing maximal response. *Pair t-test* revealed that the concentration response curves are significantly different ($P<0.05$, n = 4-5).

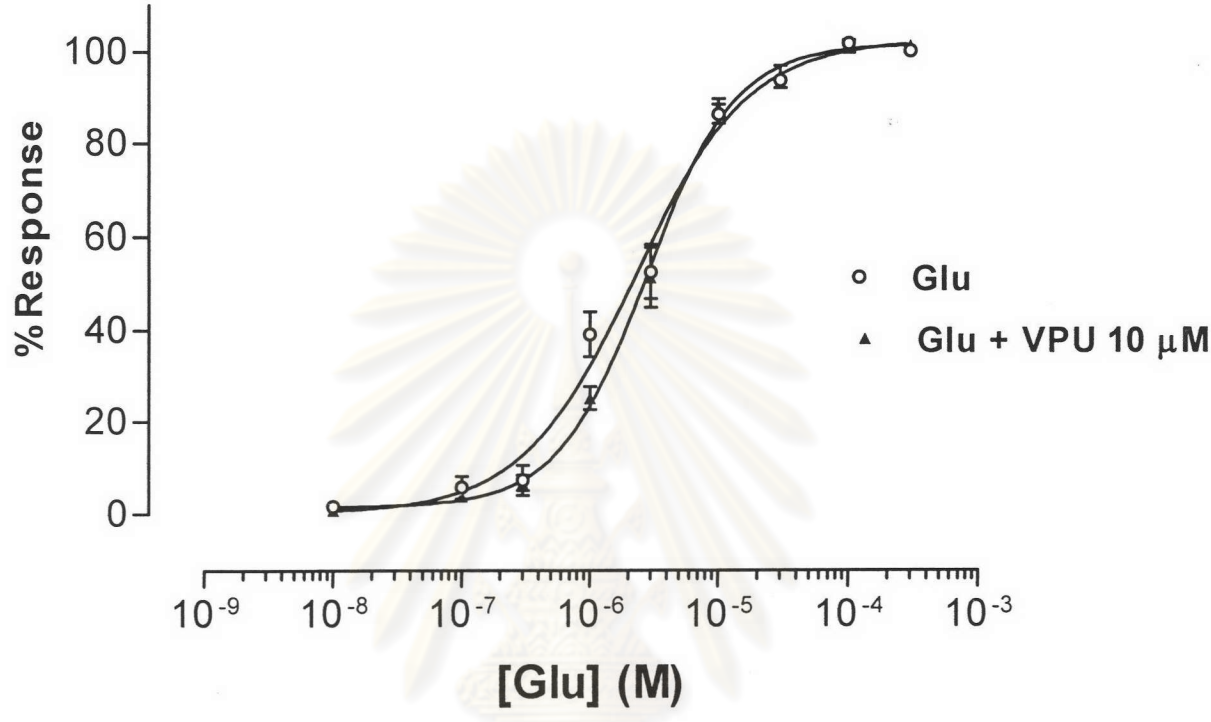

Figure 3.4 Glutamate concentration-response curves in the presence or absence of 10μM VPU. Each point is the mean ± S.E.M. of the current response of 4-6 oocytes, expressed as percentage of the maximal response. VPU produced no changes in either maximum response or Hill slope.(P> 0.05).

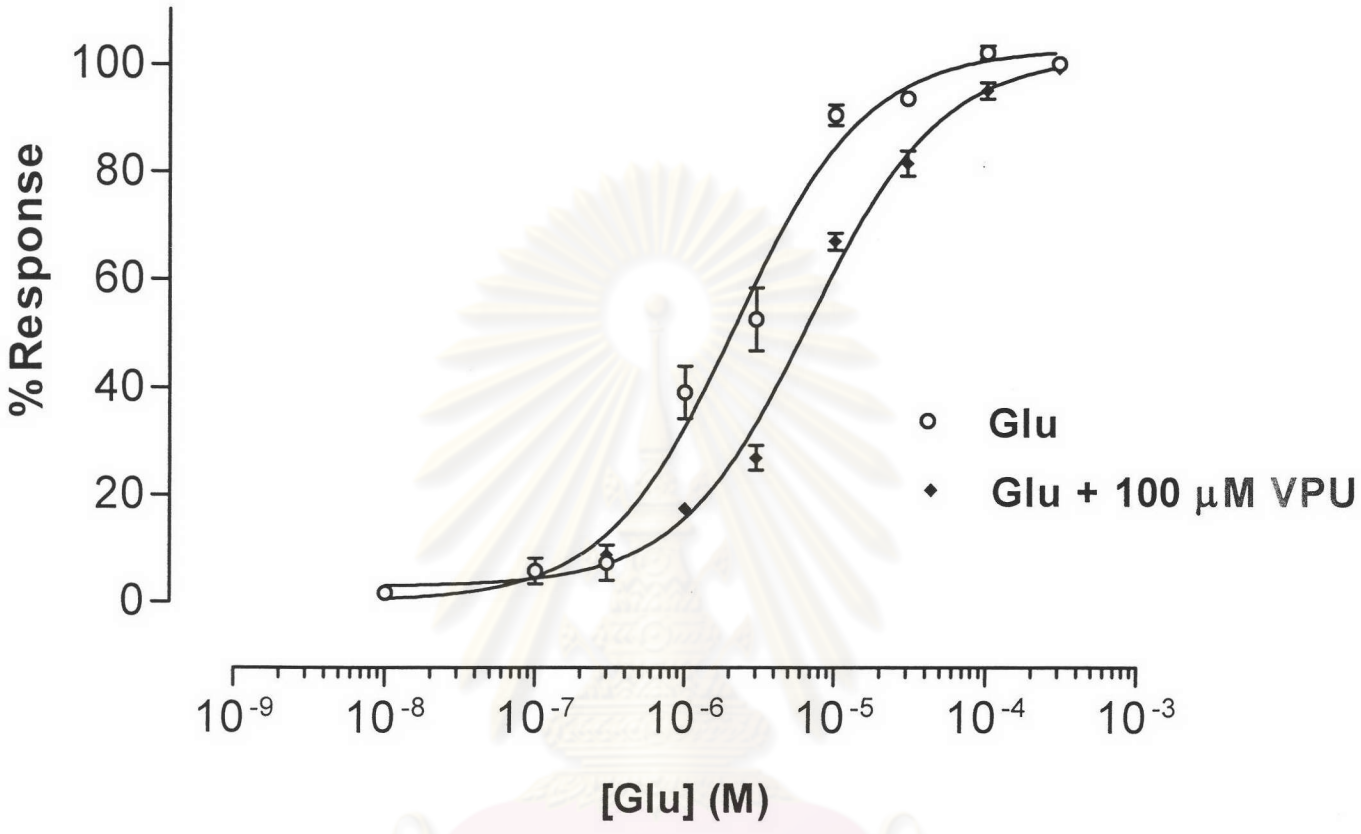

Figure 3.5 Inhibition effects of 100 μM VPU on glutamate log concentration-response curves . Each point is the mean ± S.E.M. of the current response of 4-6 oocytes, expressed as percentage of the maximal response. VPU shifted the glutamate concentration-response curve to the right without changing maximal response. *Pair t-test* revealed that the concentration response curves are significantly different ($P < 0.05$).

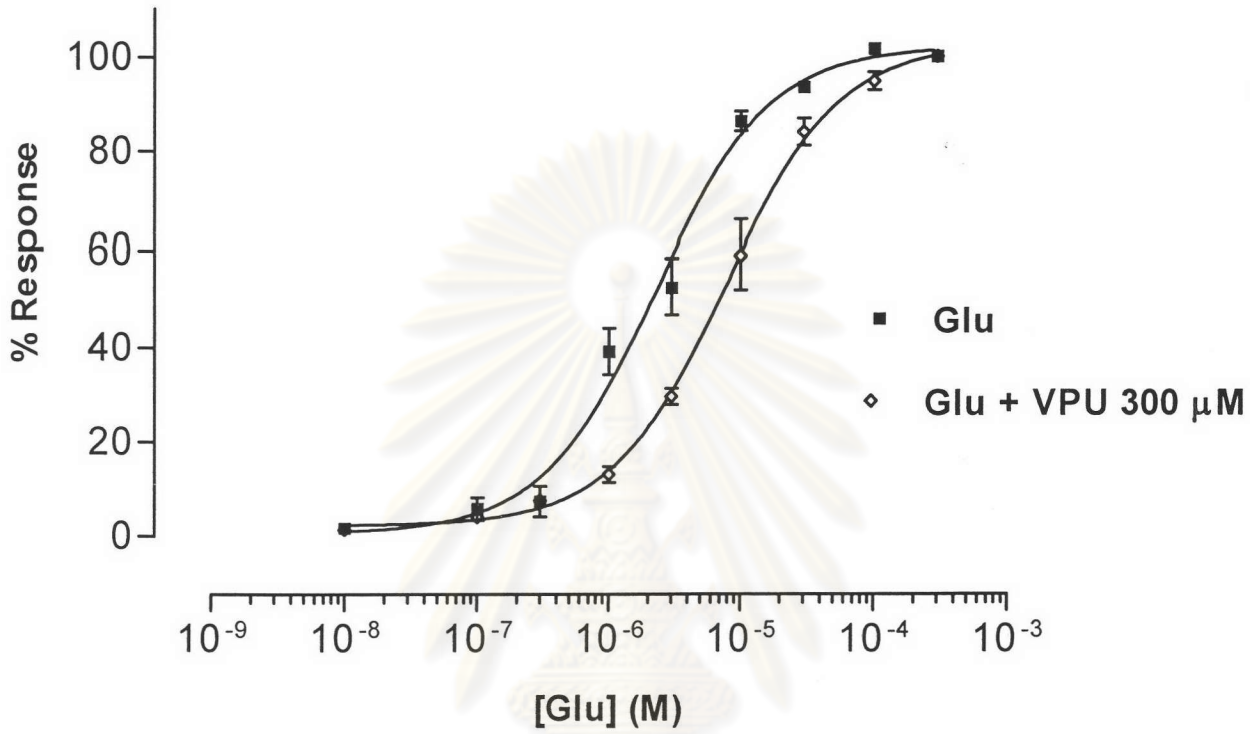

Figure 3.6 Inhibition effects of 300 μM VPU on glutamate log concentration-response curves . Each point is the mean ± S.E.M. of the current response of 4-6 oocytes, expressed as percentage of the maximal response. VPU shifted the glutamate concentration-response curve to the right without changing maximal response. *Pair t-test* revealed that the concentration response curves are significantly different ($P < 0.05$).

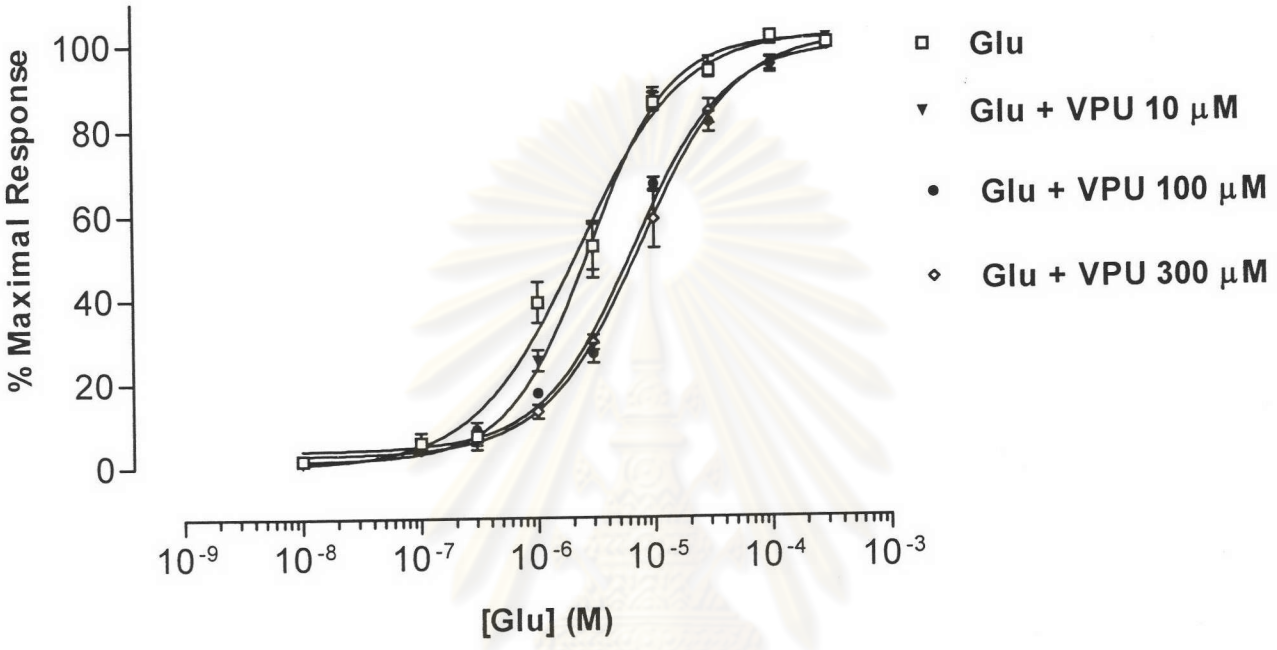

Figure 3.7 Summary of glutamate concentration-response curves demonstrating the inhibition of the NMDA receptor currents by 10 μM, 100 μM or 300 μM VPU. Each point is the mean ± S.E.M. of the current response of 4-6 oocytes, expressed as percentage of the maximal response.

	EC_{50} (μM)	Hill coefficient
Glu	2.26 ± 0.31	0.98 ± 0.12
Glu + 10 μM AP5*	5.95 ± 0.37	1.40 ± 0.10
Glu +10 μM VPU	2.79 ± 0.24	1.25 ± 0.04
Glu +100 μM VPU*	6.67 ± 0.55	1.10 ± 0.09
Glu + 300 μM VPU*	7.61 ± 0.81	1.06 ± 0.04
Glu + 1 mM VPA	2.02 ± 0.18	1.73 ± 0.21

Table 3.1 The mean EC_{50} ± S.E.M. and Hill coefficient of glutamate and glutamate with AP5, VPA and VPU calculated from log concentration response curve. * indicates statistic significance ($P < 0.05$) between control and test-substance-treated groups.

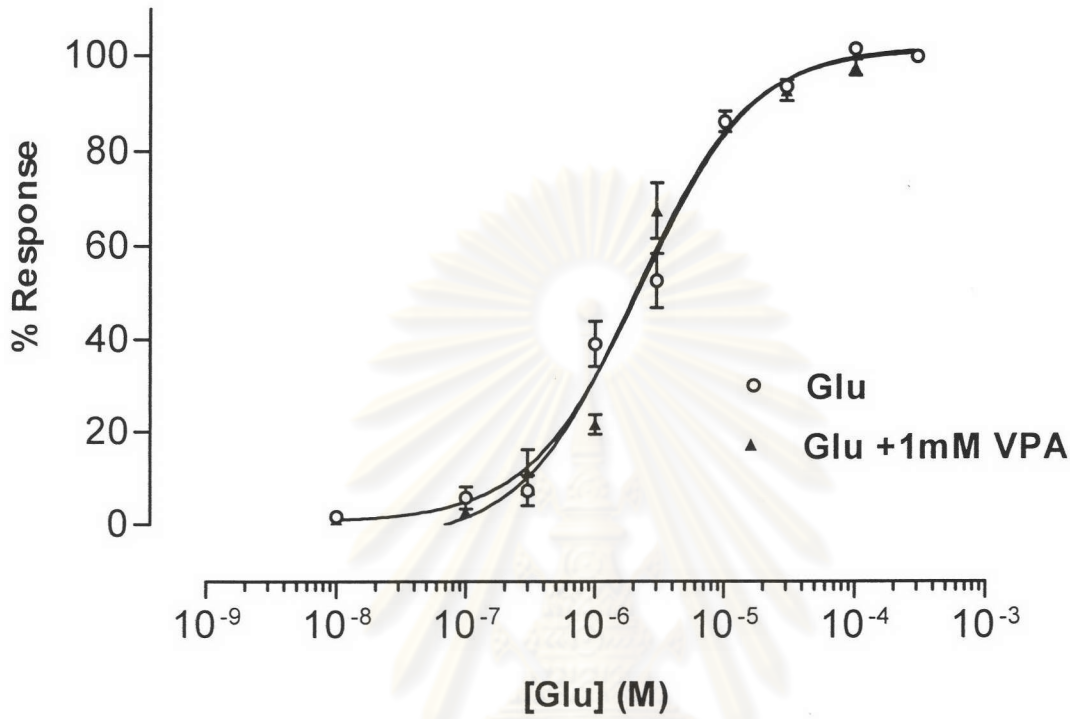

Figure 3.8 Inhibition effects of 1mM VPA on glutamate log concentration-response curves . Each point is the mean ± S.E.M. of the current response of 4-6 oocytes, expressed as percentage of the maximal response. *Pair t-test* revealed that the concentration response curves are not significantly different ($P>0.05$).

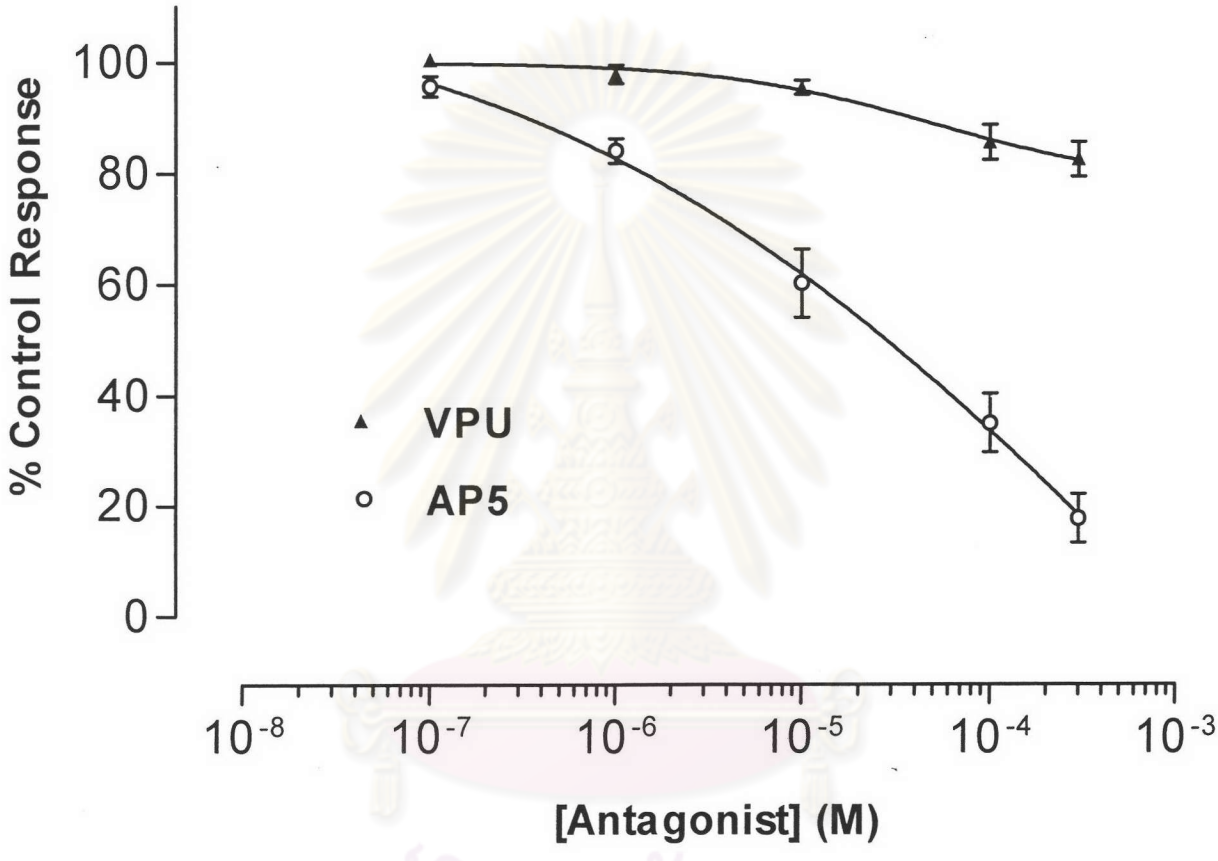

Figure 3.9 Inhibitory effects of VPU and AP5 on glutamate-induced currents. Antagonistic response curves show the inhibitions of glutamate-induced currents by 100 μM VPU and 10 μM AP5 in the presence of 3 μM glutamate and 10 μM glycine. Values were normalized to the extrapolated maximal agonist-induced current in the absence of VPU and AP5. Data points are means±SEM of the relative remaining current observed on 3–5 different oocytes and the lines are the best fit of the data points.

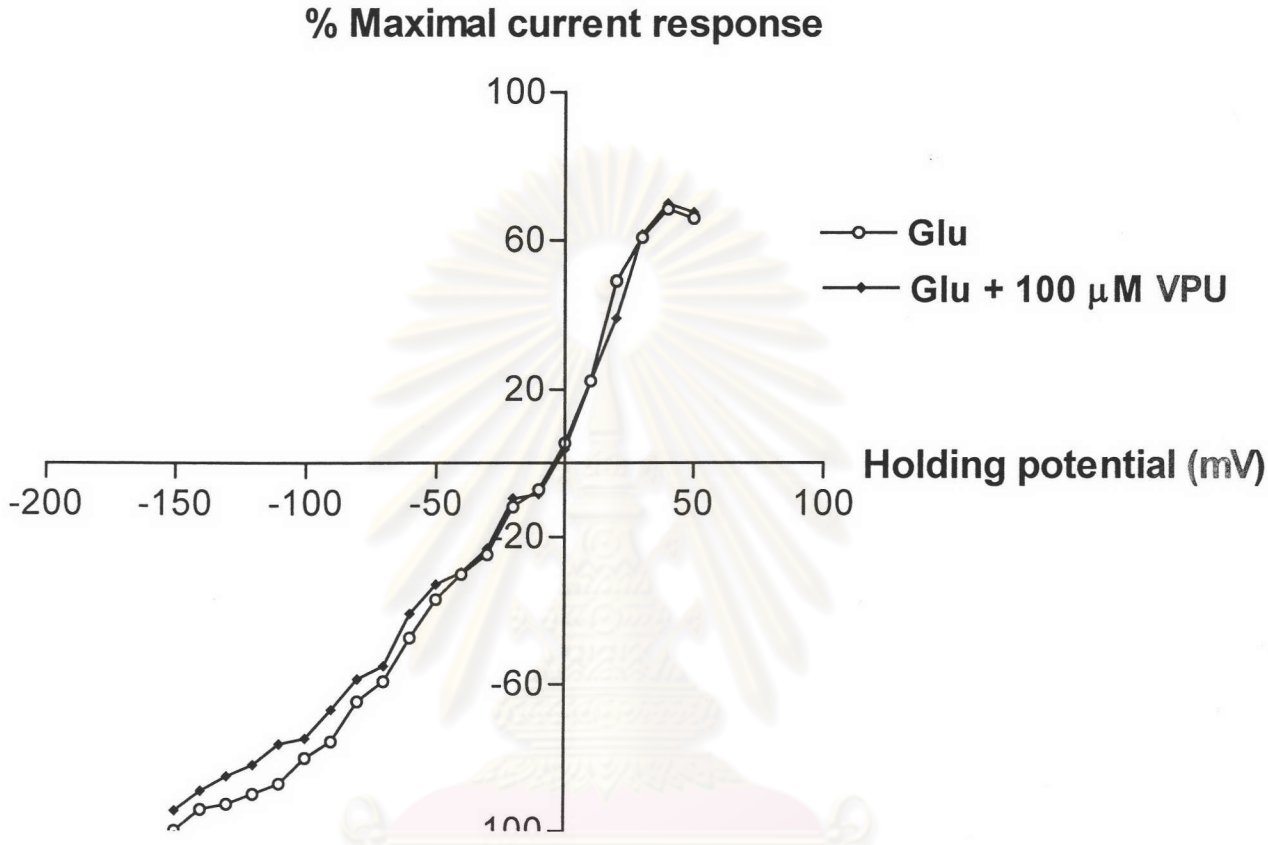

Figure 3.10 I-V relationships determined from NR1A/NR2B mRNA injected oocytes before and after application of 100 μM VPU. Each point is the mean ± S.E.M. of the current response of 2-4 oocytes, expressed as percentage of the maximal current responses.

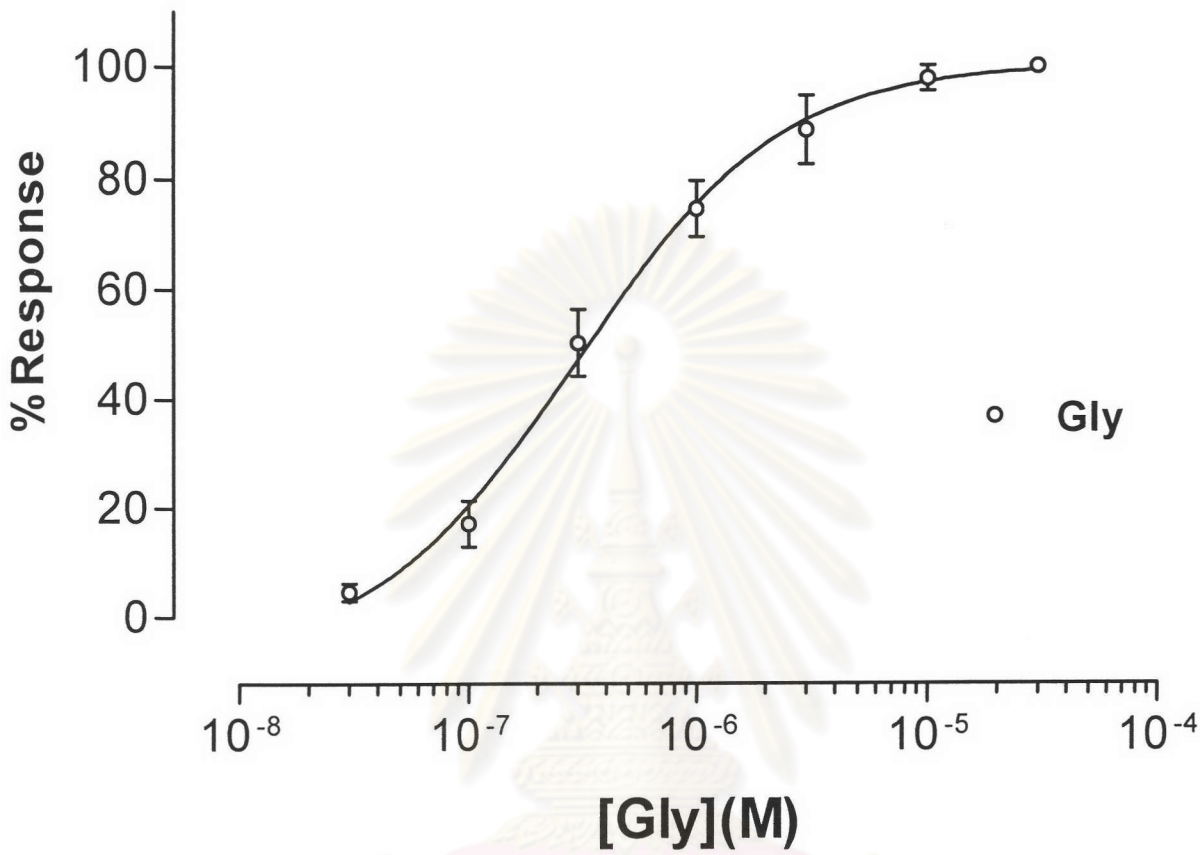

Figure 3.11 Log concentration-response relationship of glutamate (3 μM)in the presence of various concentration of glycine. Each point is the mean ± S.E.M. of the current response of 4-6 oocytes, expressed as percentage of the maximal response.

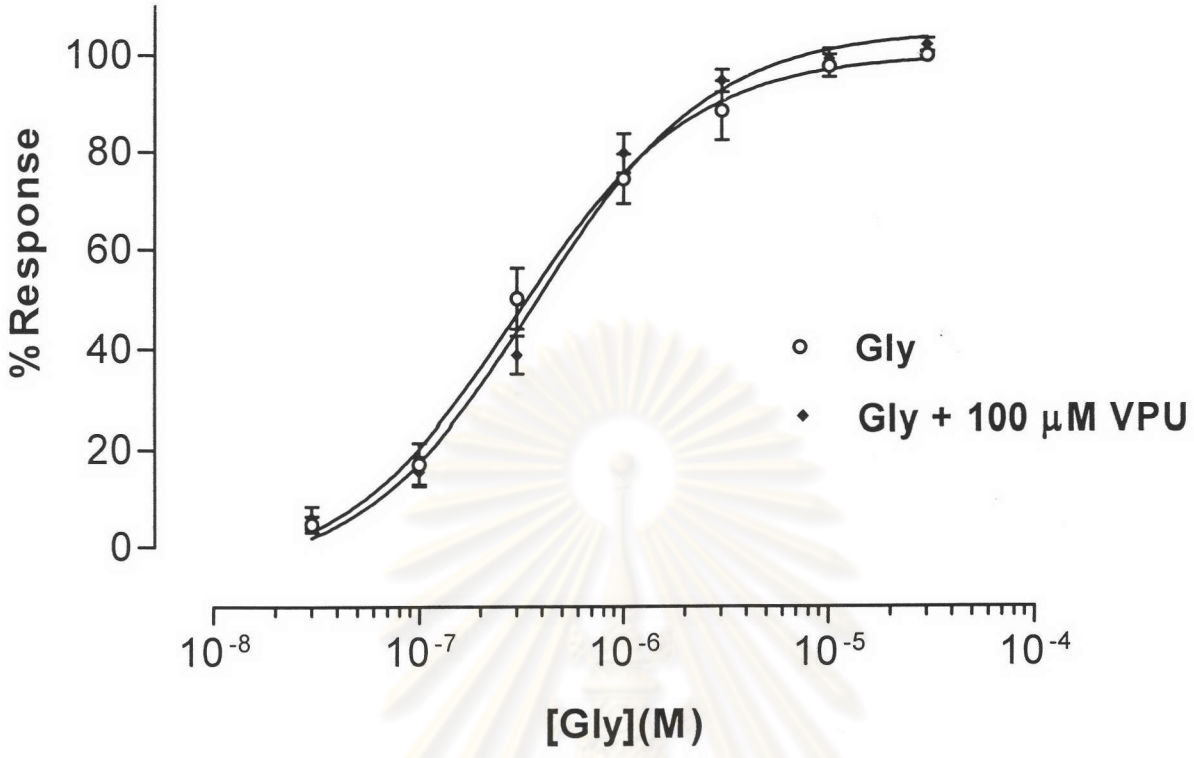

Figure 3.12 Log concentration-response relationship of glutamate (3 μM) in the presence of various concentration of glycine, and in the presence or absence of 10μM VPU. Each point is the mean ± S.E.M. of the current response of 4-6 oocytes, expressed as percentage of the maximal response. VPU produced no changes in either maximum response or Hill slope.($P > 0.05$).

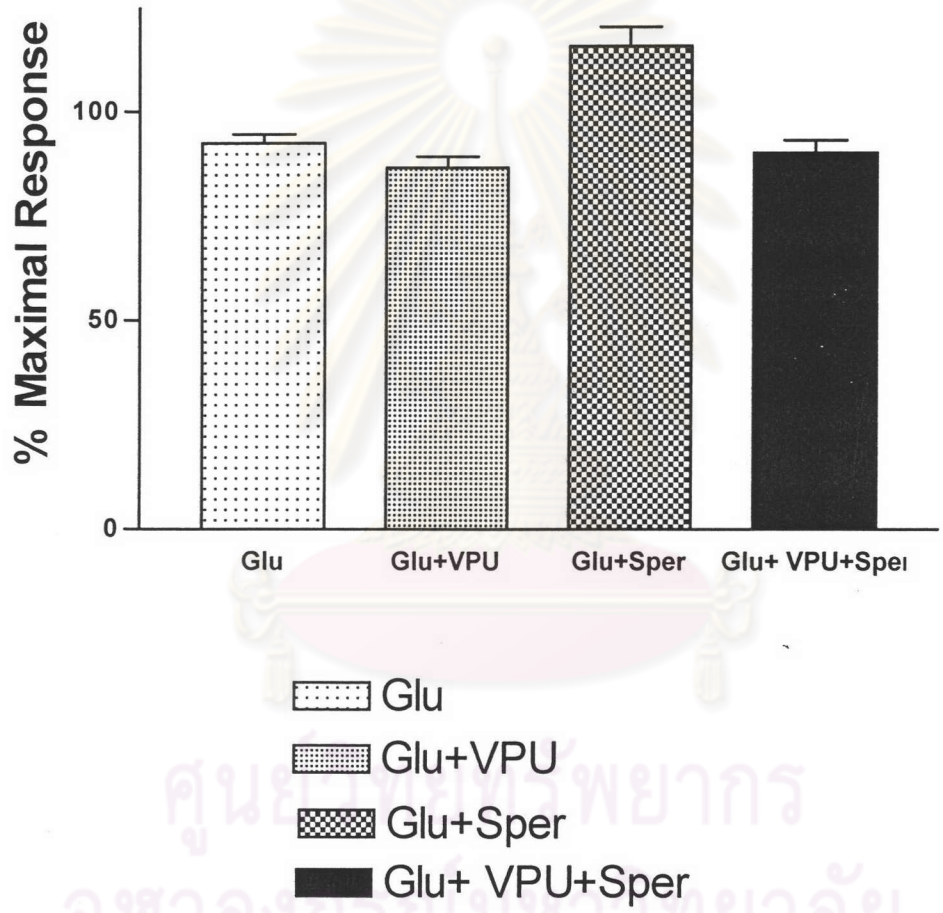

Figure 3.13 Summary of the inhibitory effects of 100 μM VPU on glutamate-induced current responses in the absence and presence of 100 μM spermine. Each column represent the mean ± S.E.M. from 3-5 different oocytes.

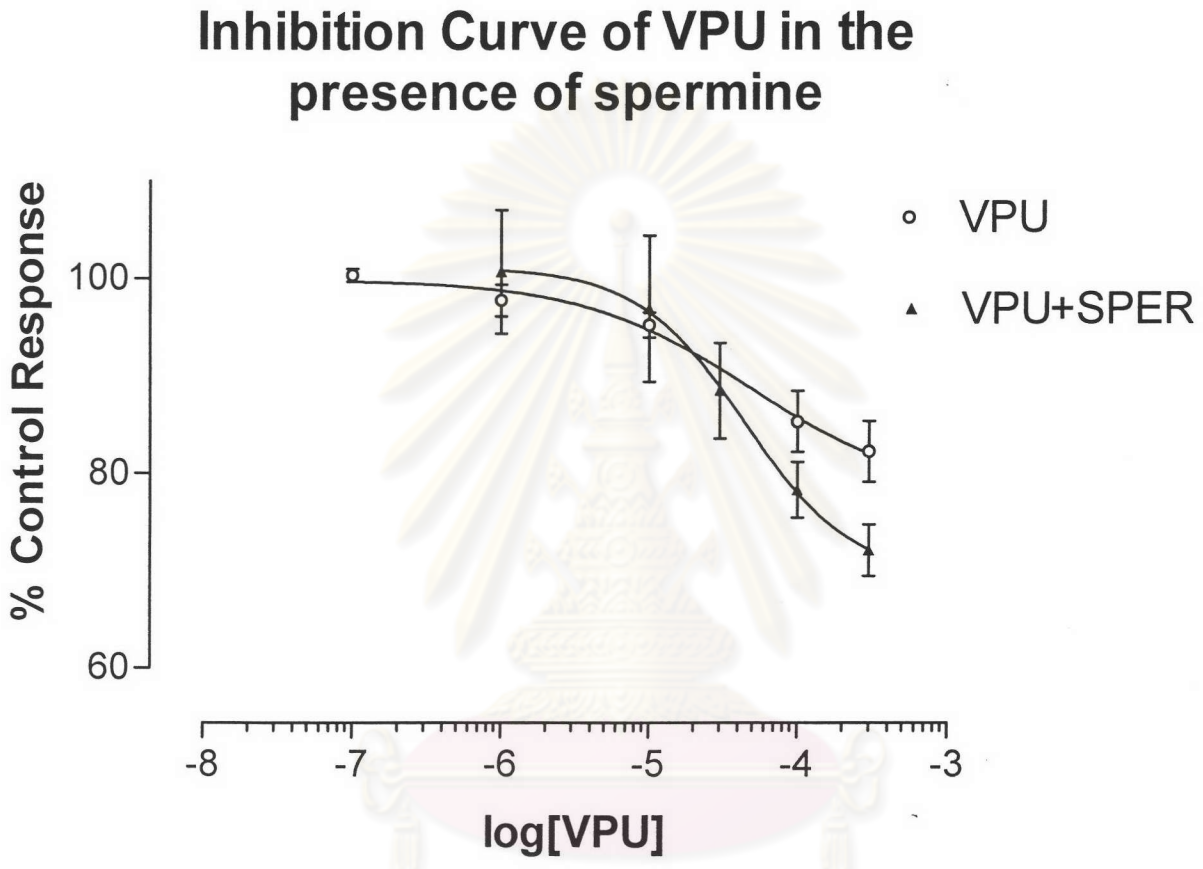

Figure 3.14 Inhibition effects of 100 μM VPU on glutamate log concentration-response curves in the absence and presence of 100 μM spermine. Each point is the mean ± S.E.M. of the current response of 4-5 oocytes, expressed as percentage of the control response in the absence and presence of spermine, respectively.

.