CHAPTER IV

RESULTS

The results were divided in to 6 parts on these following topics:-

1. The effect of CSD on leukocyte-endothelial interaction.
2. The effect of CSD on rCBF and pial arteriolar diameter.
3. The effect of NOS inhibitor on rCBF and MABP.
4. The effect of NOS inhibitor on CSD-induced cerebral hyperemia.
5. The effect of 5-HT_{1B} receptor agonist on rCBF and MABP
6. The effect of 5-HT_{1B} receptor agonist on CSD-induced cerebral hyperemia.

1. The effect of CSD on leukocyte-endothelial interaction.

The adherent leukocyte to venular endothelium was not observed in both two groups, KCl and NaCl application (show in figure 4.1). No significant leukocyte adhesion was demonstrated in this study. Because of the lake of the effect of CSD on leukocyte adhesion, no further experiment was conducted to determine the effect of L-NAME and naratriptan on this event.

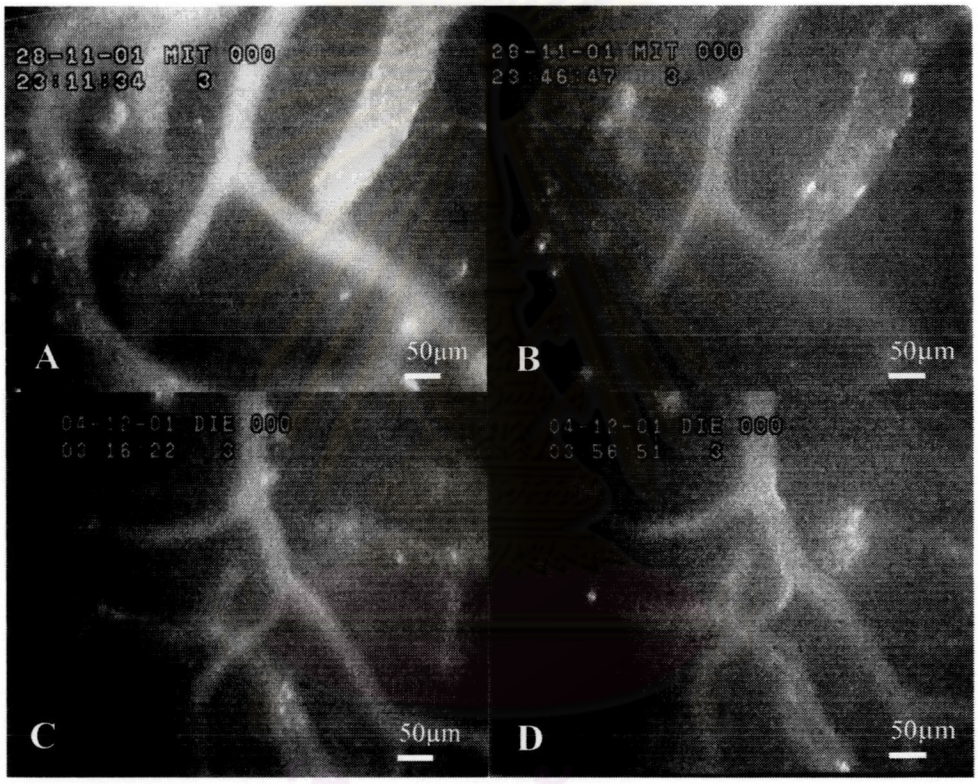

Figure 4.1 *The intravital videomicroscope image of circulating leukocyte. A) before KCl application, B) 40 minutes after KCl application, C) before NaCl application, D) 40 minutes after NaCl application. Bar=50 μm.*

2. The effect of CSD on rCBF and pial arteriolar diameter.

2.1 *Effect on rCBF.*

Application of KCl induced the repeated pattern of cerebral hyperemia. The first hyperemia peak developed within 4 minutes after KCl application. The amplitude from each peak was calculated as percent changes from the resting stage (show in figure 4.3). The magnitude was maximum in the first peak (270% from baseline) and then decreases gradually. The median number of hyperemic cycles within one hour was 12 (vary 10 to 14). The average duration of these cycles was 4.2$\pm$0.7 minutes. On the other hand, the rCBF was not changed in the NaCl application group. Statistical analysis showed significant difference between the two groups. ($p<0.05$, ANOVA for repeated measurement) (Figure 4.3)

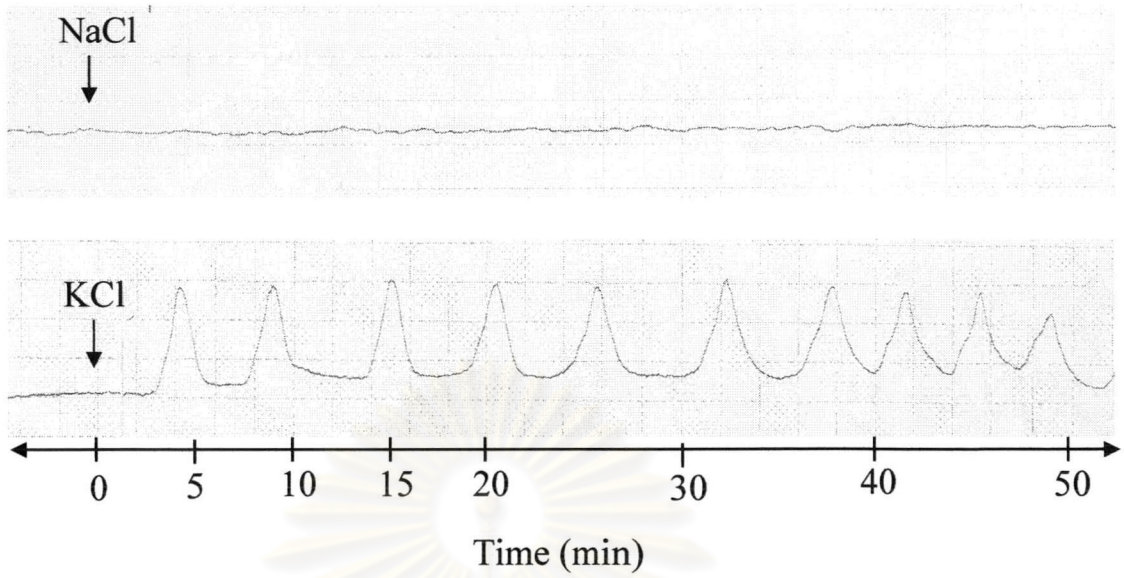

Figure 4.2 *The tracing showing the rCBF changes in NaCl application group (upper) and KCl application group (lower).*

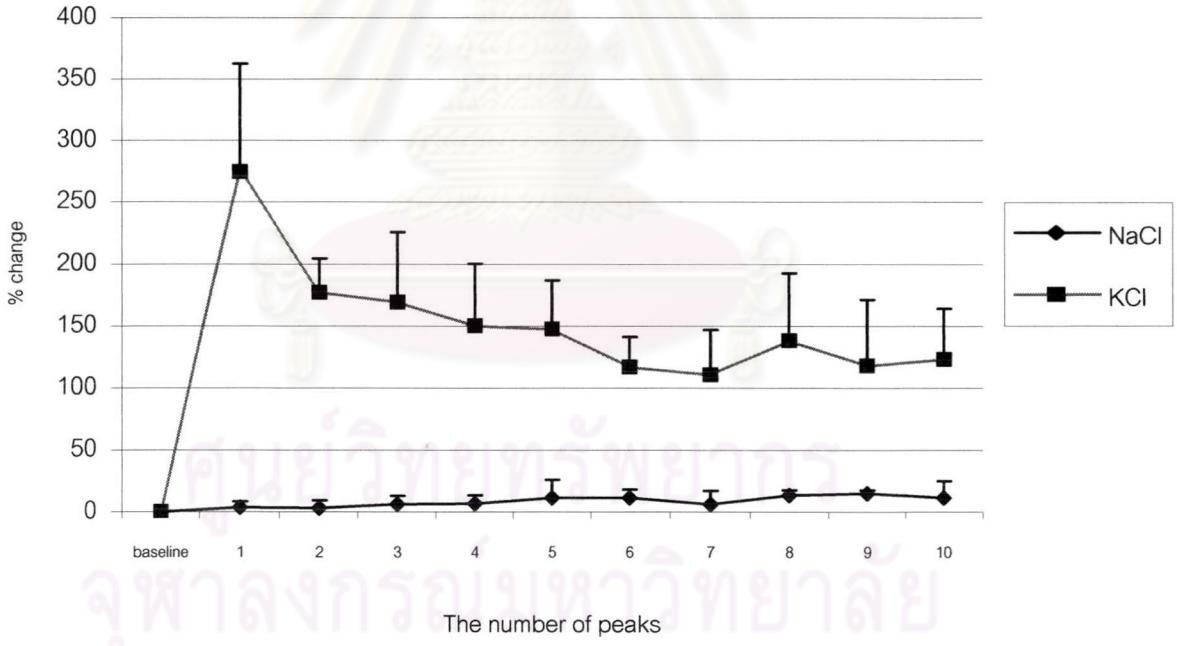

Figure 4.3 *The percent change from baseline in rCBF of NaCl and KCl application group. Significant effects were assessed using ANOVA with post hoc Dunnett's t-test treated NaCl application group as control. ($p<0.05$)*

1.2 *Effect on pial arteriolar diameter*

In KCl application group, the intravital videomicroscopy showed a repeated pattern of pial arteriolar dilation and constriction (Figure 4.4). At 3 minute after KCl application, the pial arteriole became dilated to the maximum dilation. The arteriolar dilation persisted for a few minute and then the vessels became constricted to the baseline. The duration of dilation-constriction cycles were 4.6±0.7 minutes. The pattern of these cycles correlated well with the rCBF changes measured by the laser Doppler flowmetry. On the other hand, in the NaCl application group, the pial arteriolar diameters were not changed in any period (Figure 4.5). Statistical analysis demonstrated that KCl application induced significant changes in percent change from baseline in pial arteriolar diameter as compare with NaCl application. ($p<0.05$, ANOVA for repeated measurement) (Figure 4.6)

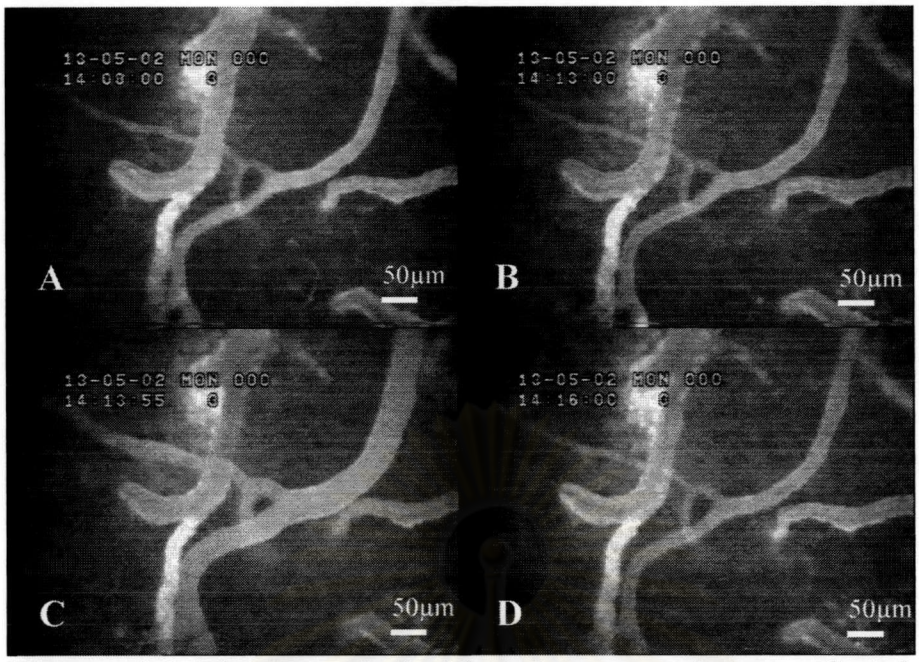

Figure 4.4 *The intravital videomicroscope image of pial arterioles before and after KCl application. A) before KCl application, B) 3 minute after KCl application, C) 4 minute after KCl application, D) 6 minute after KCl application. Significant dilation of pial arteriole was demonstrated. Bar=50 μm.*

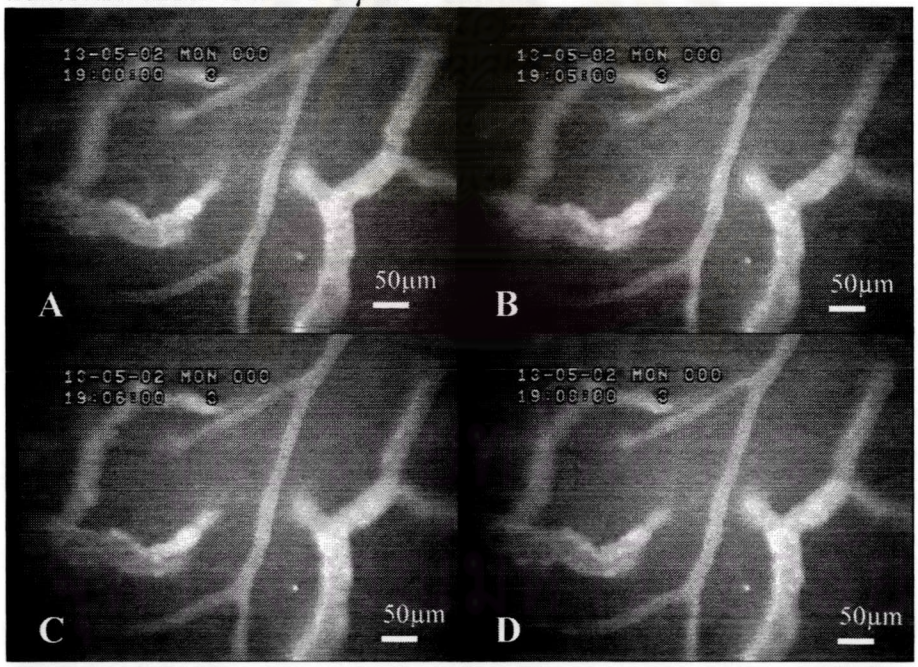

Figure 4.5 *The intravital videomicroscope image of pial arterioles before and after NaCl application. A) before NaCl application, B) 3 minute after NaCl application, C) 4 minute after NaCl application, D) 6 minute after NaCl application. No significant difference of pial arteriolar diameter was obserbed. Bar=50 μm.*

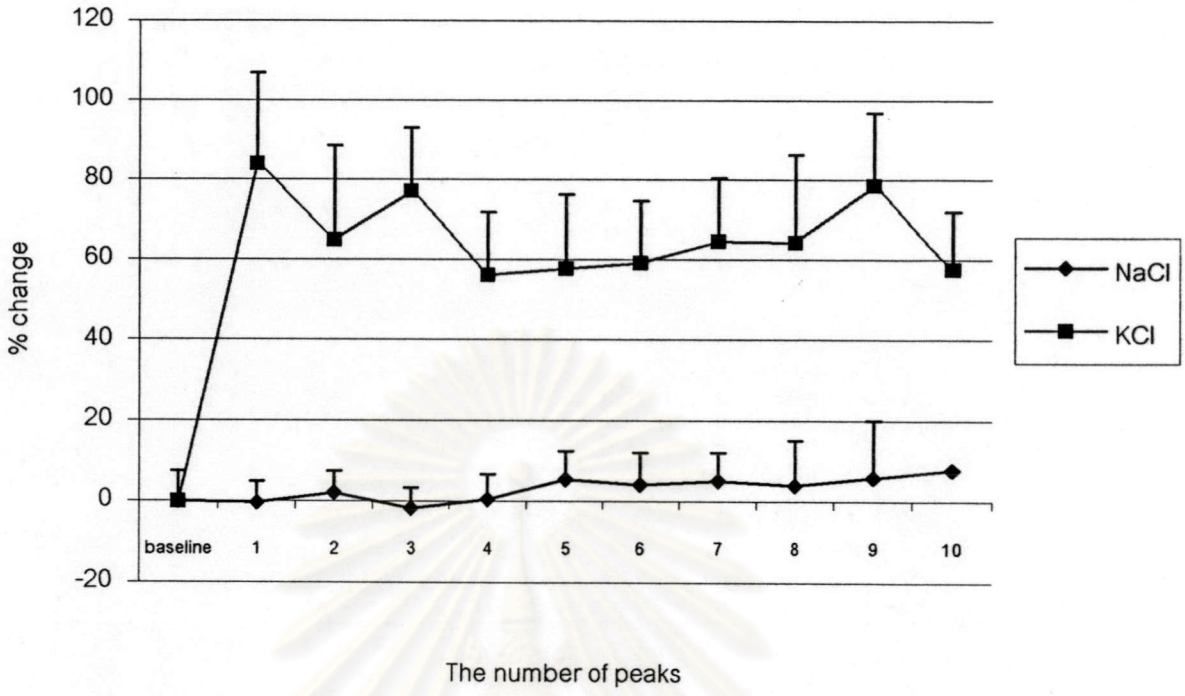

Figure 4.6 *The percent change of pial arteriolar diameter from baseline in NaCl and KCl application groups. Significant effects were assessed using ANOVA with post hoc Dunnett's t-test treated NaCl application group as control. ($p<0.05$)*

2. The effect of NOS inhibitor on rCBF and MABP.

2.1 Effect on rCBF.

In the control group, administration of either NSS or L-NAME at 10 minutes after NaCl application did not induce any changes of the rCBF in any period (Figure 4.7). Statistical analysis did not show significant difference between the two groups. ($p>0.05$) (Figure 4.8)

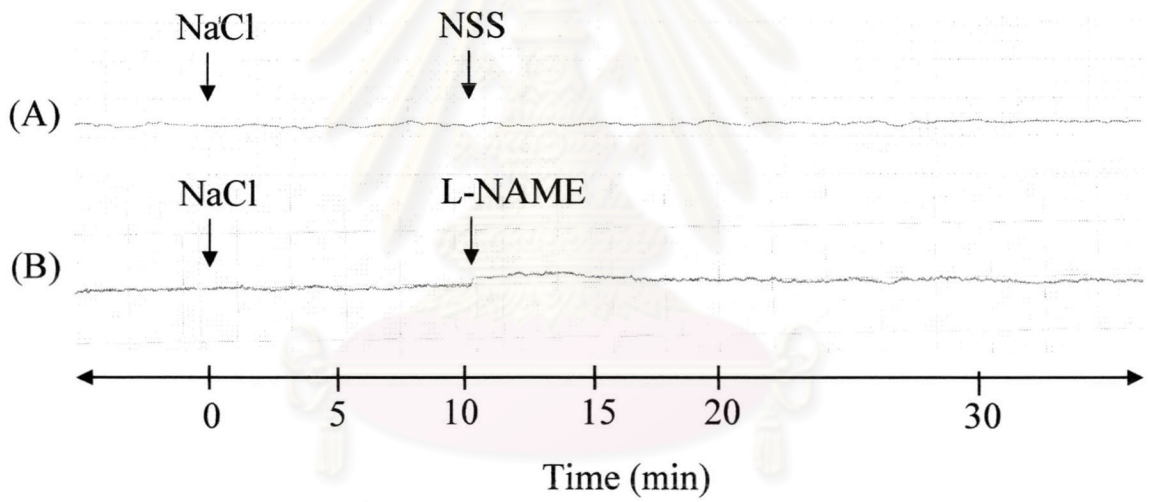

Figure 4.7 *The tracing showing the example of the rCBF changes in NaCl application rats without L-NAME treatment (A) and with L-NAME treatment (B).*

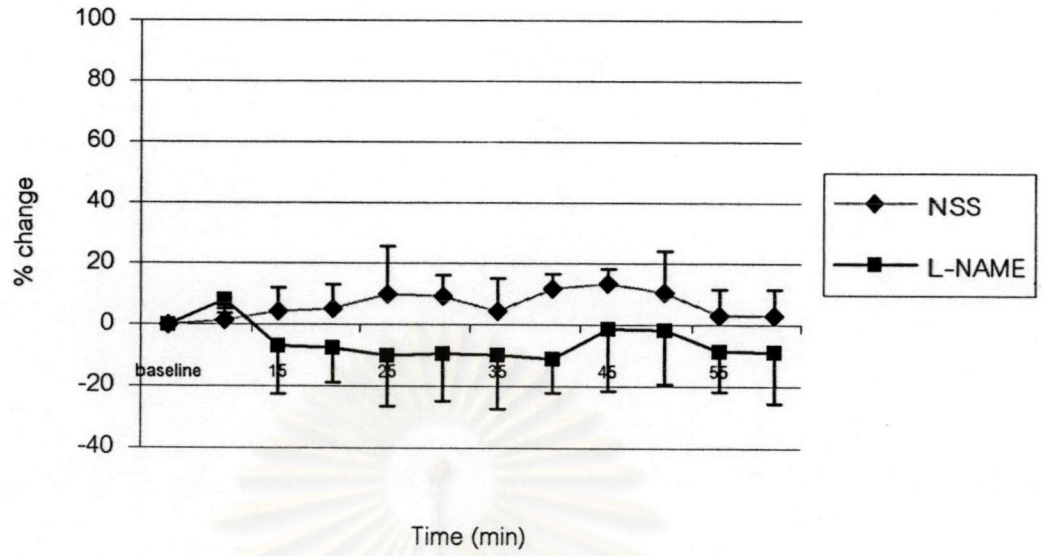

Figure 4.8 *The percent change of rCBF from baseline NaCl application rats with and without L-NAME treatment. No significant difference between two groups was observed. ($p>0.05$, ANOVA for repeated measurement)*

2.2 *Effect on MABP*

In the control group, the MABP remained stable throughout the experiment (Figure 4.9). The calculated MABP at 5 minutes before NaCl application to 60 minutes after NaCl application were showed in figure 4.10.

On the other hand, a rapid decrease in the MABP was observed in the L-NAME-treated group, followed by the increasing of both systolic and diastolic blood pressure (Figure 4.9). However, the difference in percent change from baseline in MABP was not significant as compare with NSS treated group. ($p>0.05$) (Figure 4.10)

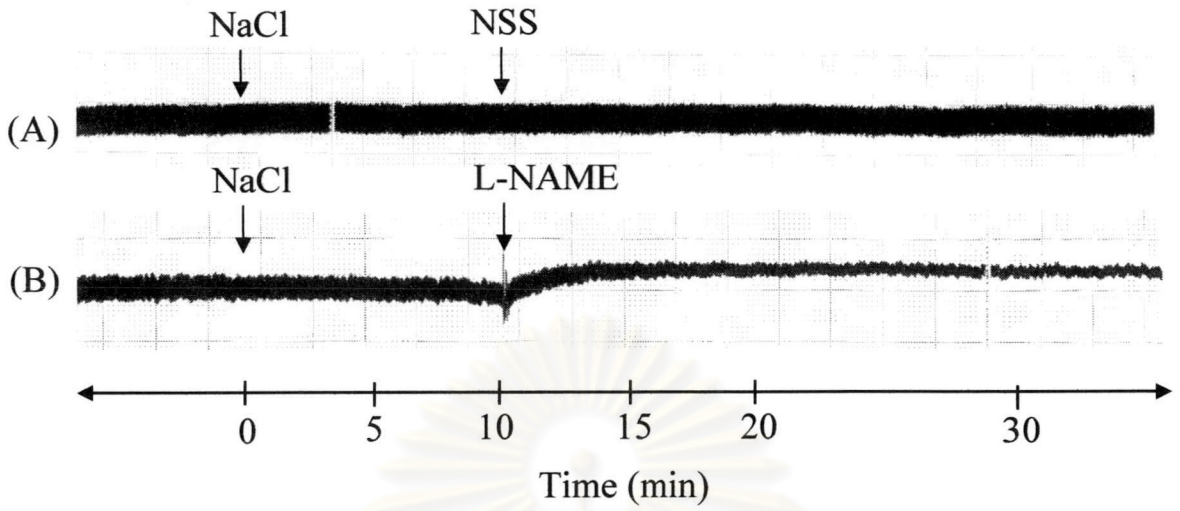

Figure 4.9 *The tracing showing the MABP changes in NaCl application rats without L-NAME treatment (A) and with L-NAME treatment (B).*

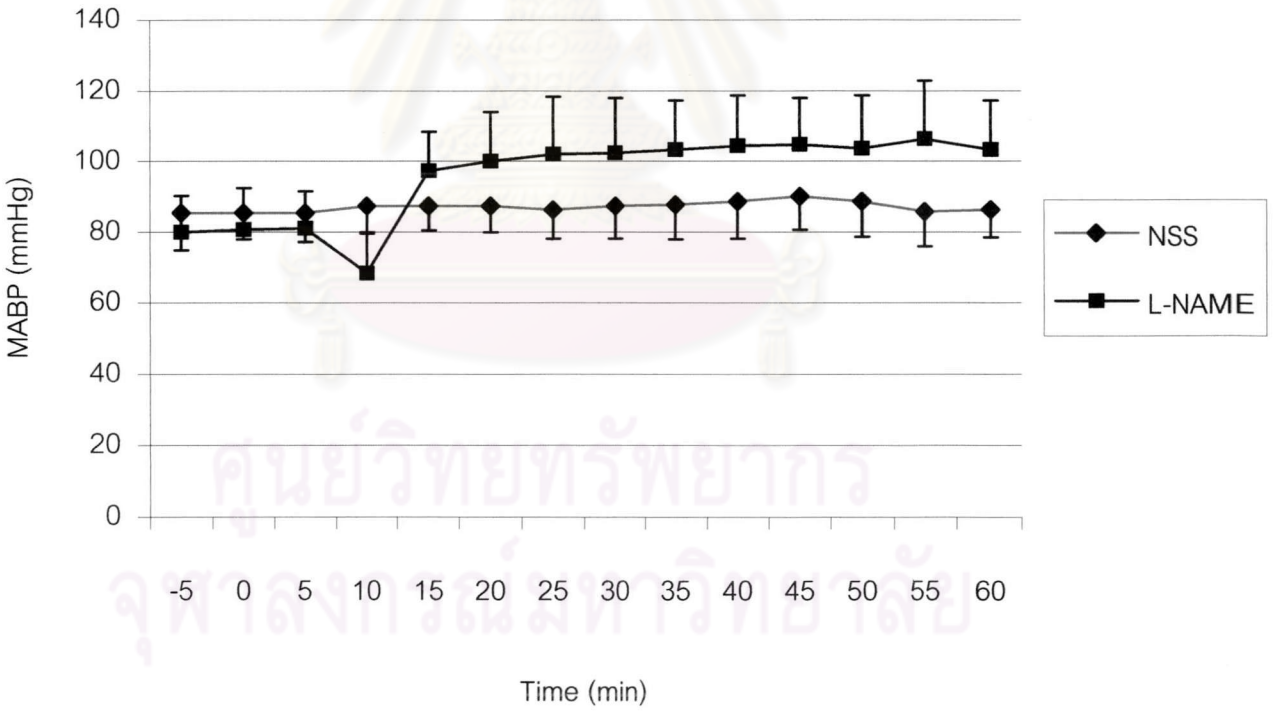

Figure 4.10 *The percent change from baseline in MABP of NaCl application rats with and without L-NAME treatment. No significant difference between two groups was observed. (p>0.05, ANOVA for repeated measurement)*

3. **The effect of NOS inhibitor on CSD-induced cerebral hyperemia.**

3.1 *Effect on rCBF.*

The results showed that administration of L-NAME after the 2nd hyperemic cycle decreased the amplitude of hyperemic peak. The decreasing of hyperemic peaks was demonstrated at the peaks after treatment and was significantly observed after the 4th peak (Figure 4.11). The percent reduction was calculated from each peak after treatment compared with the peaks before treatment (Figure 4.13). Statistical analysis demonstrated that administration of NOS inhibitor could significantly reduce the hyperemic changes in rCBF as compare with KCl induced CSD group. ($p < 0.05$) (Figure 4.12, 4.13)

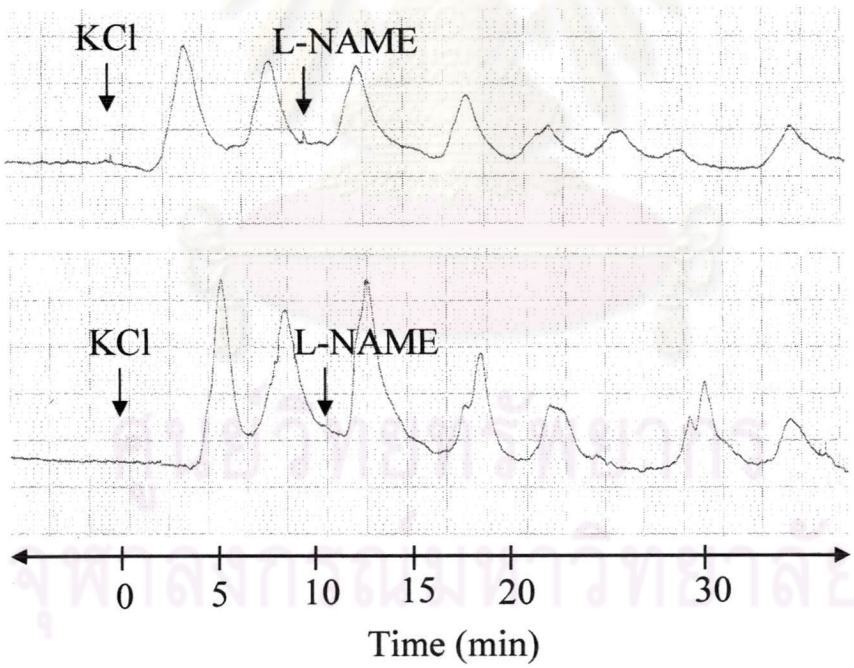

Figure 4.11 *The tracing showing two example of the rCBF changes in KCl application rats with L-NAME treatment (both upper and lower).*

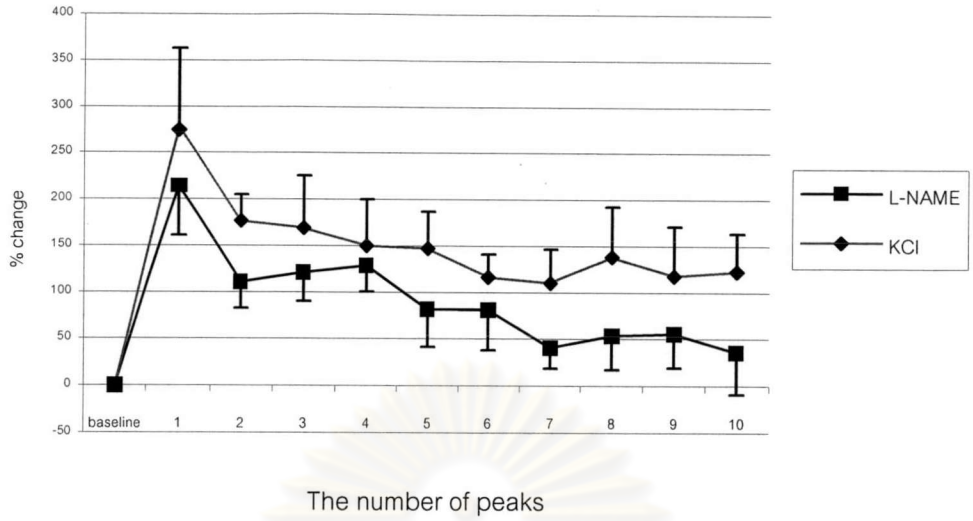

Figure 4.12 *The percent change of rCBF from baseline in KCl application rats with and without L-NAME treatment. Significant effects were assessed using ANOVA with post hoc Dunnett's t-test treated KCl application without L-NAME-treated group as control. (p <0.05)*

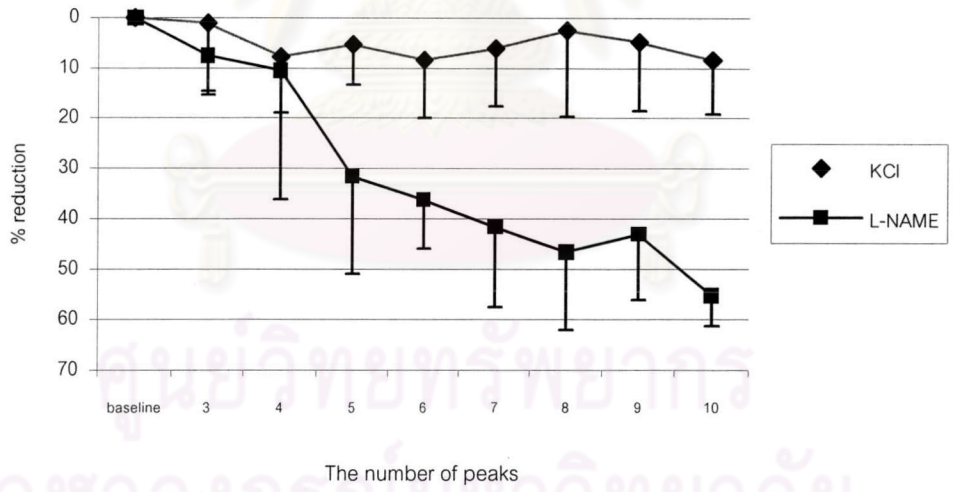

Figure 4.13 *The percent reduction of rCBF from baseline in KCl application rats with and without L-NAME treatment. Significant effects were assessed using ANOVA with post hoc Dunnett's t-test treated KCl application without L-NAME-treated group as control. (p <0.05)*

Effect of L-NAME on CSD-evoked hyperemia: Dose-effect relationship

NOS inhibitor, L-NAME, was injected intravenously in various doses after the 2nd hyperemic cycle in the KCl application group. The dosages of L-NAME were selected in 3 doses at 1, 10 and 100 mg/kg BW.

Administration of L-NAME at the dose of 1 mg/kg BW did not clearly decrease the amplitude of hyperemic peaks, substantially (Figure 4.14). It was not significant difference when compared with the KCl application group ($p>0.05$). An administration of L-NAME at the dose of 100 mg/kg BW almost completely decreased the amplitude of hyperemic peaks and eventually abolished these cycles (Figure 4.14). However, it was not significant difference when compared with the L-NAME-treated group at the dose of 10 mg/kg BW. ($p< 0.05$)

We also found that the reduction of the amplitude of hyperemic peaks by L-NAME administration in KCl application group was dose dependent manner.

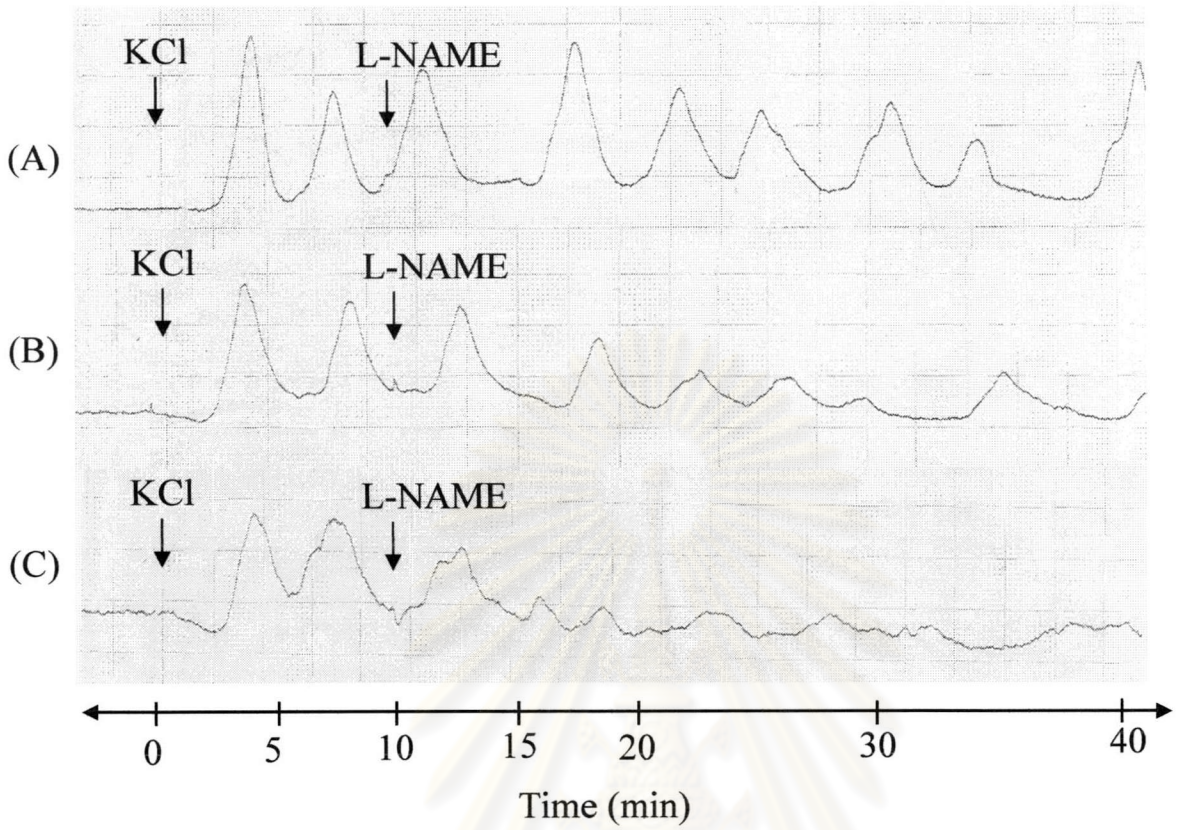

Figure 4.14 *The tracing showing the rCBF changes in KCl application rats with L-NAME treatment. (A) at the dose of 1 mg/kg BW, (B) at the dose of 10 mg/kg BW, (C) at the dose of 100 mg/kg BW.*

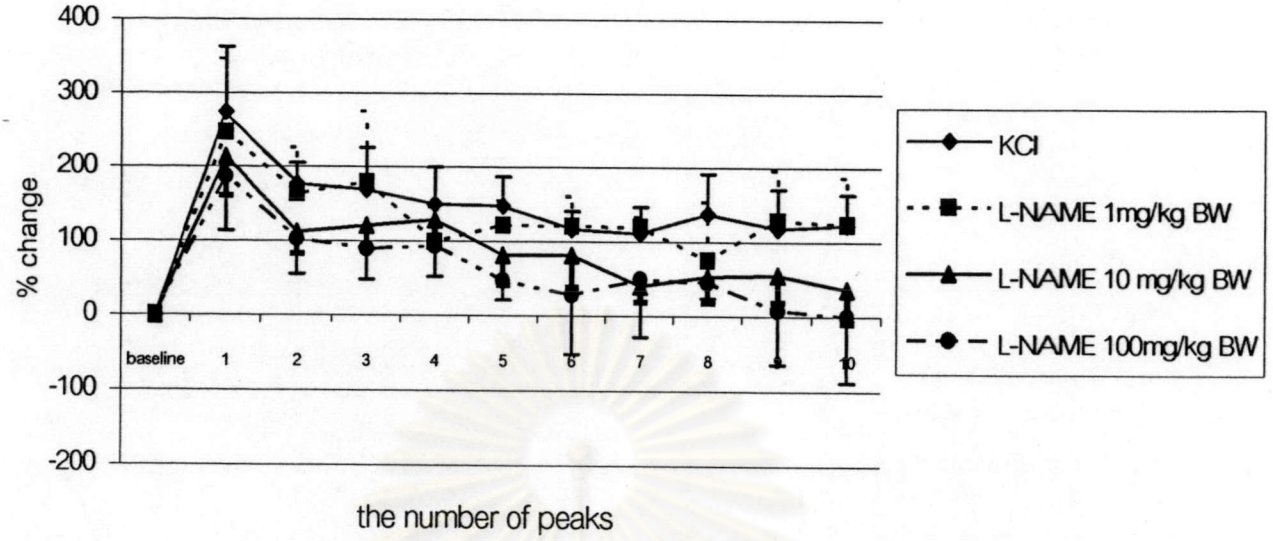

Figure 4.15 *The percent change of rCBF from baseline in KCl application with L-NAME-treated group: Dose-effect relationship.*

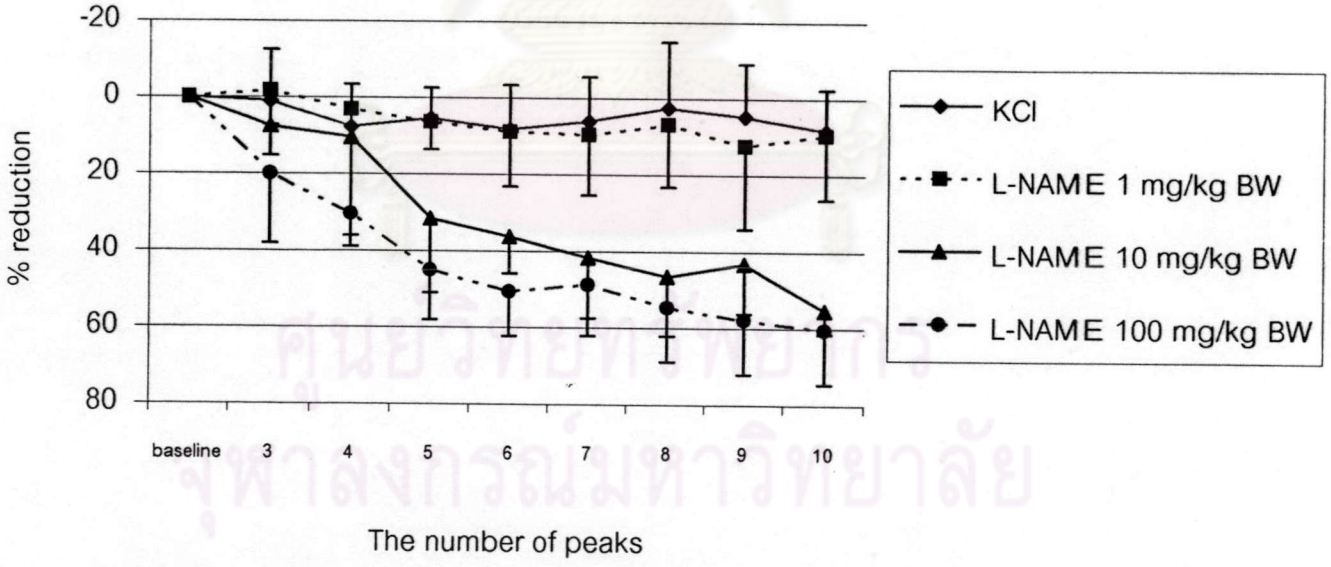

Figure 4.16 *The percent reduction of rCBF from baseline in KCl application with L-NAME-treated group: Dose-effect relationship.*

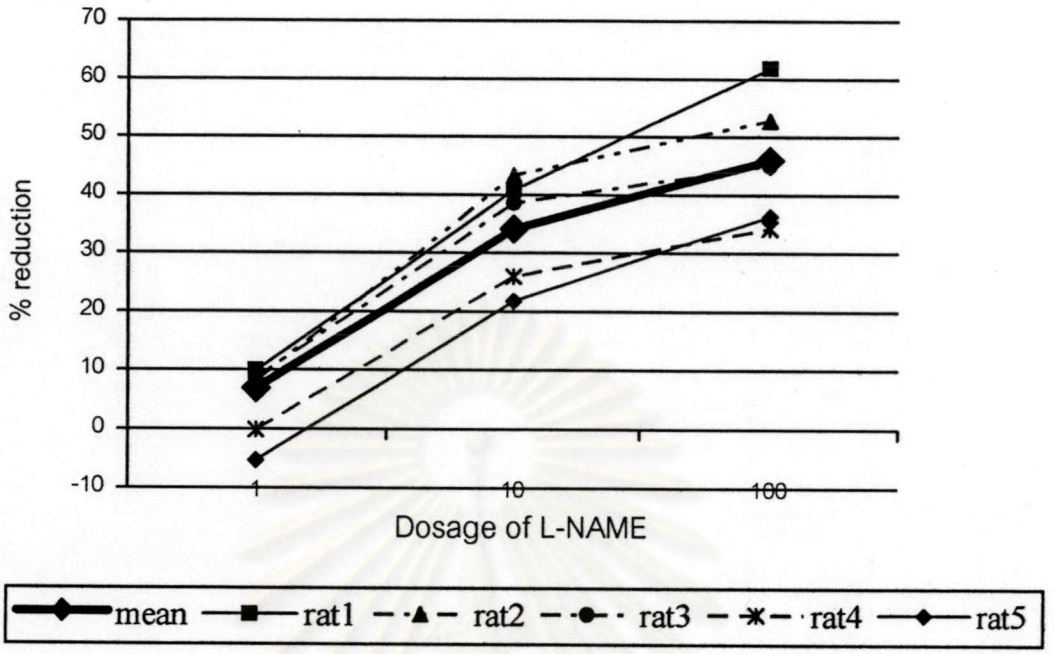

Figure 4.17 *The effect of L-NAME on CSD-evoked cerebral hyperemia in each rat: Dose-effect relationship.*

3.2 *Effect on pial arteriolar diameter*

The results showed that administration of L-NAME 10 mg/ kg BW after the 2nd hyperemic cycle decreased the vasodilation of hyperemic peaks. The intravital videomicroscopy showed that the maximum vasodilations of hyperemic peaks were minimized in the peaks after treatment. These effects of L-NAME administration correlated well with the effect on rCBF measured by the laser Doppler flowmetry. The arteriolar diameter in KCl application with L-NAME-treated group showed a significant different difference in the percent change and percent reduction from baseline as compare with KCl application without L-NAME-treated group. ($p<0.05$, ANOVA for repeated measurement) (Figure 4.19, 4.20)

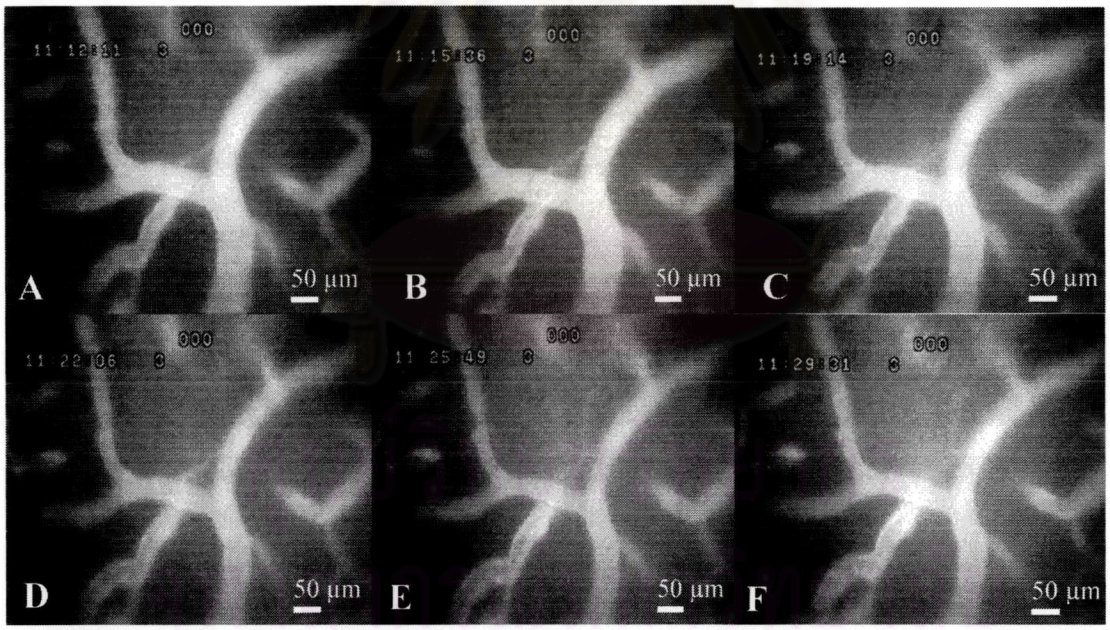

Figure 4.18 *The intravital videomicroscope image of pial arterioles at the maximum vasodilation in each peak in the KCl application rats with L-NAME treatment. A, B, C, D, E and F showing at the 1st, 2nd, 3rd, 4th, 5th and 6th peak, respectively. Bar=50 µm.*

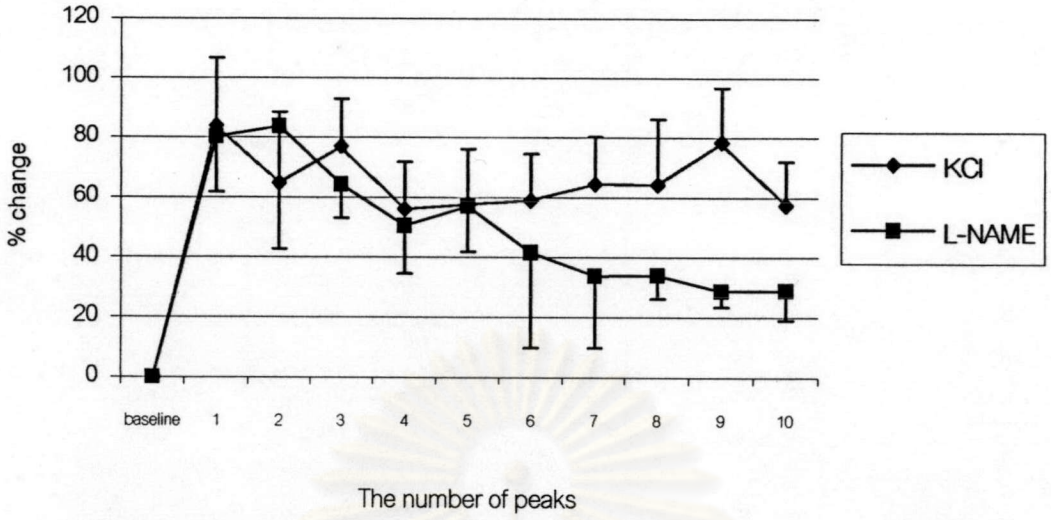

Figure 4.19 *The percent change of pial arteriolar diameter from baseline in KCl application rats with and without L-NAME treatment. Significant effects were assessed using ANOVA with post hoc Dunnett's t-test treated KCl application without L-NAME-treated group as control. ($p<0.05$)*

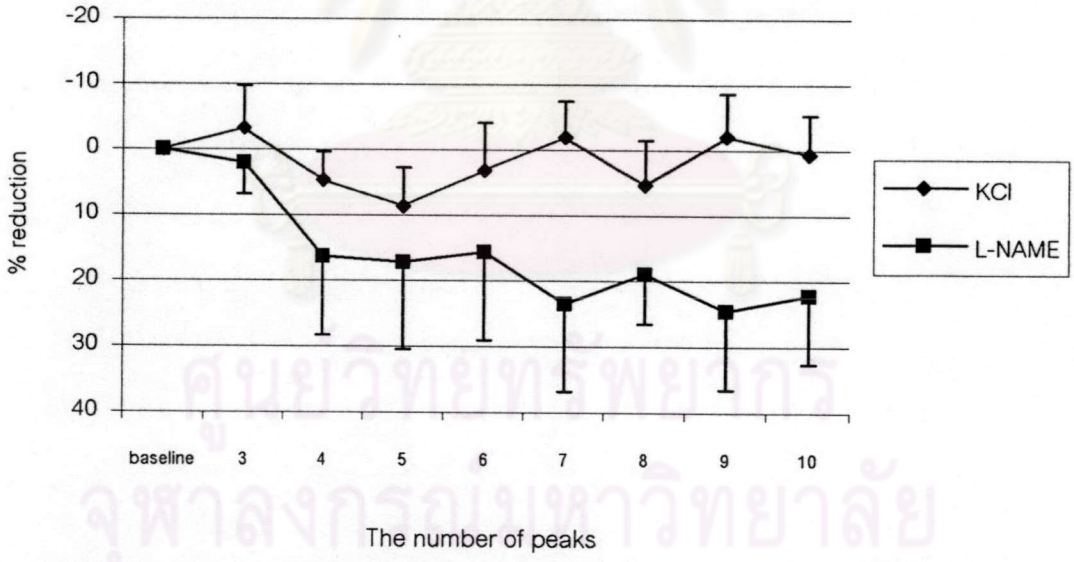

Figure 4.20 *The percent reduction of pial arteriolar diameter from baseline in KCl application rats with and without L-NAME treatment. Significant effects were assessed using ANOVA with post hoc Dunnett's t-test treated KCl application without L-NAME-treated group as control. ($p<0.05$)*

4. The effect of 5-HT_{1B} receptor agonist on rCBF and MABP.

4.1 Effect on rCBF.

In the control group, naratriptan were injected intravenously at 10 minutes after NaCl application at the dose of 0.1 mg/kg BW. The results demonstrated that either NSS or naratriptan administration had no effect on rCBF in any period. (Figure 4.21)

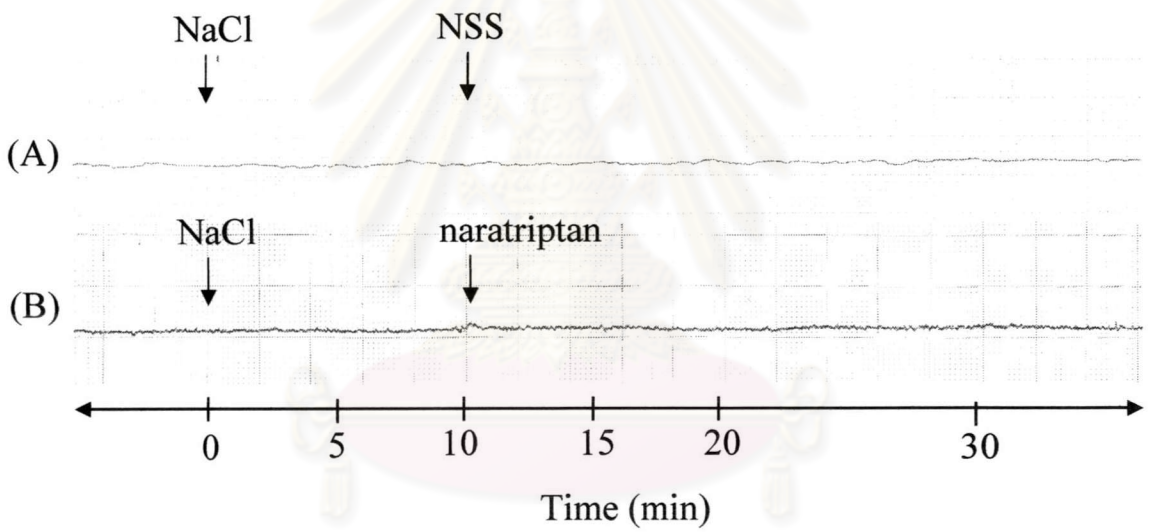

Figure 4.21 *The tracing showing the rCBF changes in NaCl application rats without naratriptan treatment (A) and with naratriptan treatment (B).*

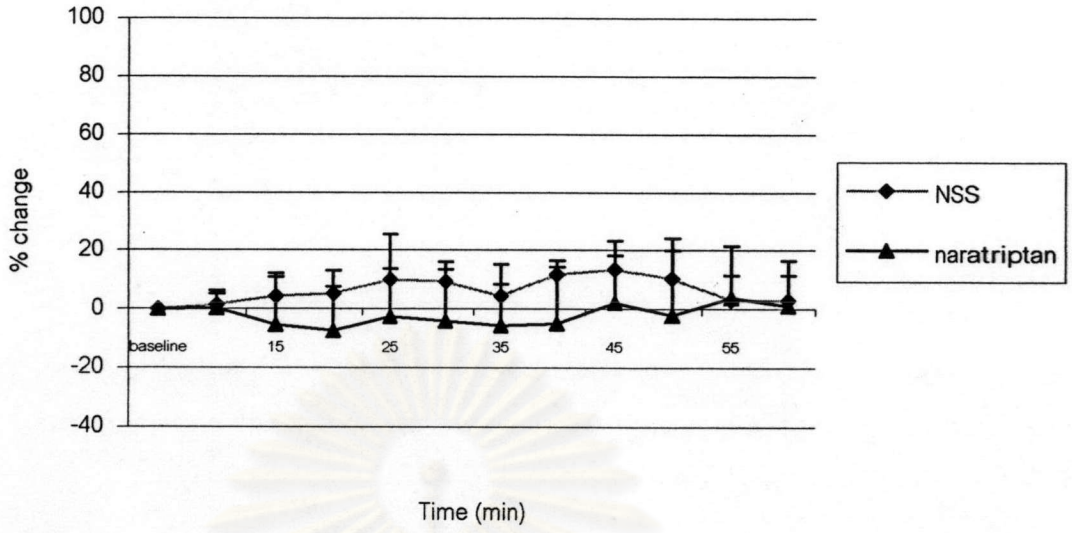

Figure 4.22 *The percent change of rCBF from baseline in NaCl application rats with and without naratriptan treatment. No significant difference between two groups was observed. (p>0.05, ANOVA for repeated measurement)*

4.2 *Effect on MABP*

In the control group, the MABP remained stable throughout the experiment (Figure 4.23). The calculated MABP at 5 minutes before NaCl application to 60 minutes after NaCl application were showed in figure 4.24.

In the naratriptan-treated group, the MABP also rapidly decreased after treatment and then became normalized throughout the experiment. The difference was not statistically significant as compared with the control group. ($p>0.05$) (Figure 4.24)

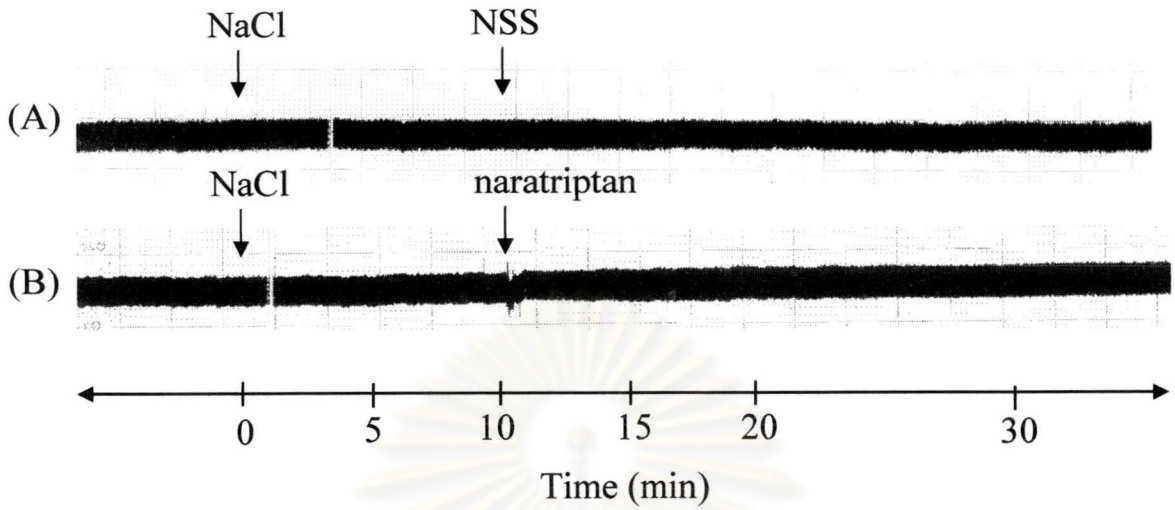

Figure 4.23 *The tracing showing the MABP changes in NaCl application rats without naratriptan treatment (A) and with naratriptan treatment (B).*

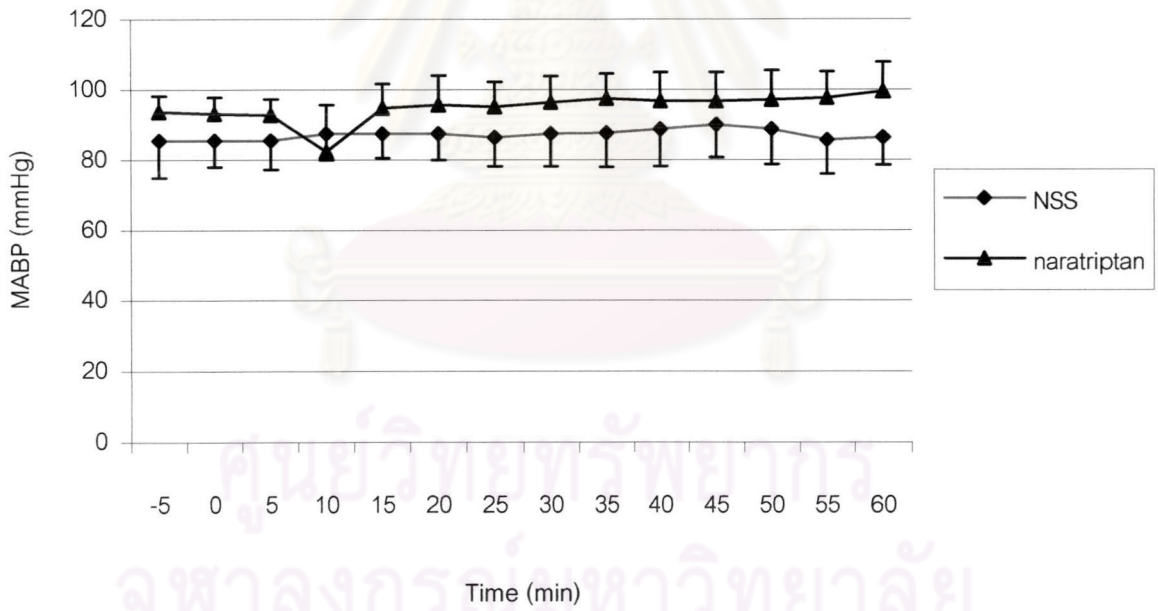

Figure 4.24 *The percent change of MABP from baseline in NaCl application rats with and without naratriptan treatment. No significant difference between two groups was observed. ($p>0.05$, ANOVA for repeated measurement)*

5. The effect of 5-HT_{1B} receptor agonist on CSD-induced cerebral hyperemia.

5.1 *Effect on rCBF.*

5-HT_{1B} receptor agonist, naratriptan, was injected intravenously at the dose of 0.1 mg/kg BW after the 2nd hyperemic cycle in the KCl application group. The results showed that administration of naratriptan did not clearly decrease the amplitude of hyperemic peaks. Statistical analysis demonstrated that both percent change and percent reduction from baseline in rCBF had no significant difference between the KCl application with and without naratriptan-treated group. ($p>0.05$, ANOVA for repeated measurement) (Figure 4.26, 4.27)

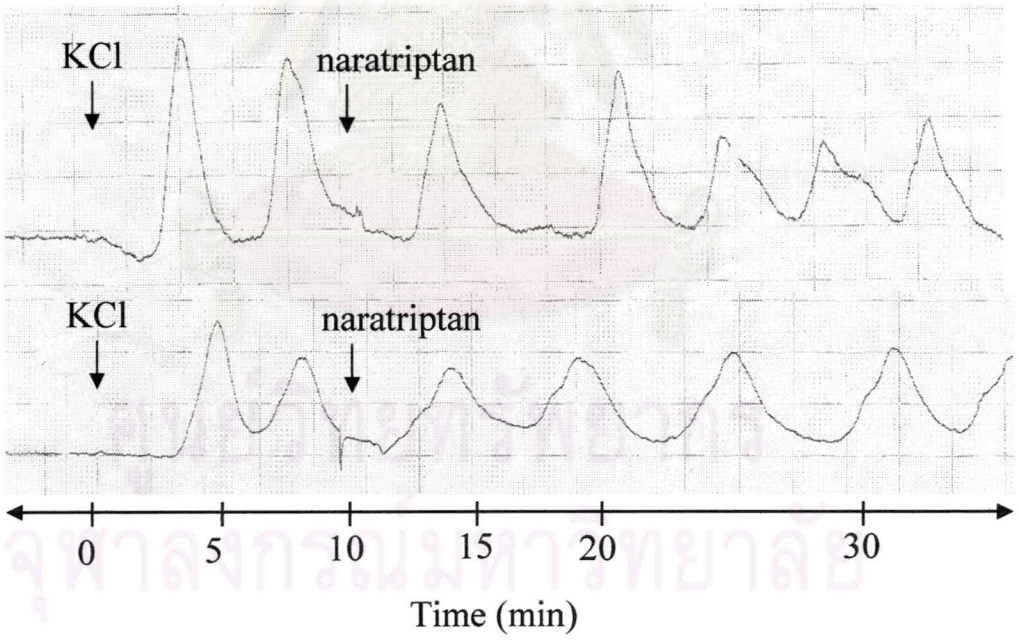

Figure 4.25 *The tracing showing the rCBF changes in KCl application group with naratriptan treatment (both upper and lower).*

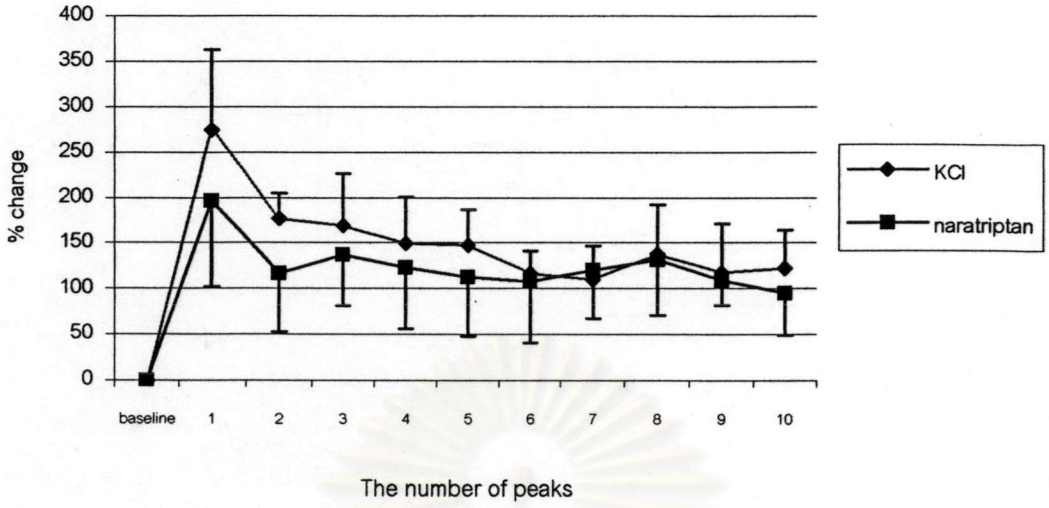

Figure 4.26 *The percent change of rCBF from baseline in KCl application rats with and without naratriptan treatment. No significant difference between two groups was observed. (p>0.05, ANOVA for repeated measurement)*

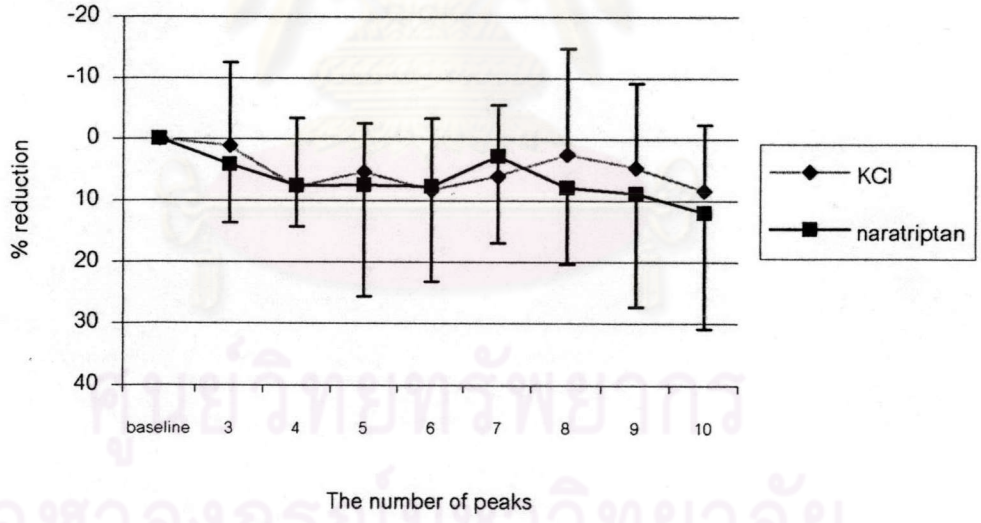

Figure 4.27 *The percent reduction of rCBF from baseline in KCl application rats with and without L-NAME treatment. No significant difference between two groups was observed. (p>0.05, ANOVA for repeated measurement)*

5.2 *Effect on pial arteriolar diameter*

5-HT_{1B} receptor agonist, naratriptan, was injected intravenously at the dose of 0.1 mg/kg BW after the 2nd hyperemic cycle in the KCl application group. Administration of naratriptan did not clearly decrease the vasodilation of hyperemic peak (Figure 4.28). The percent change and percent reduction from baseline did not show a significant difference between those two groups. ($p>0.05$, ANOVA for repeated measurement) (Figure 4.29, 4.30)

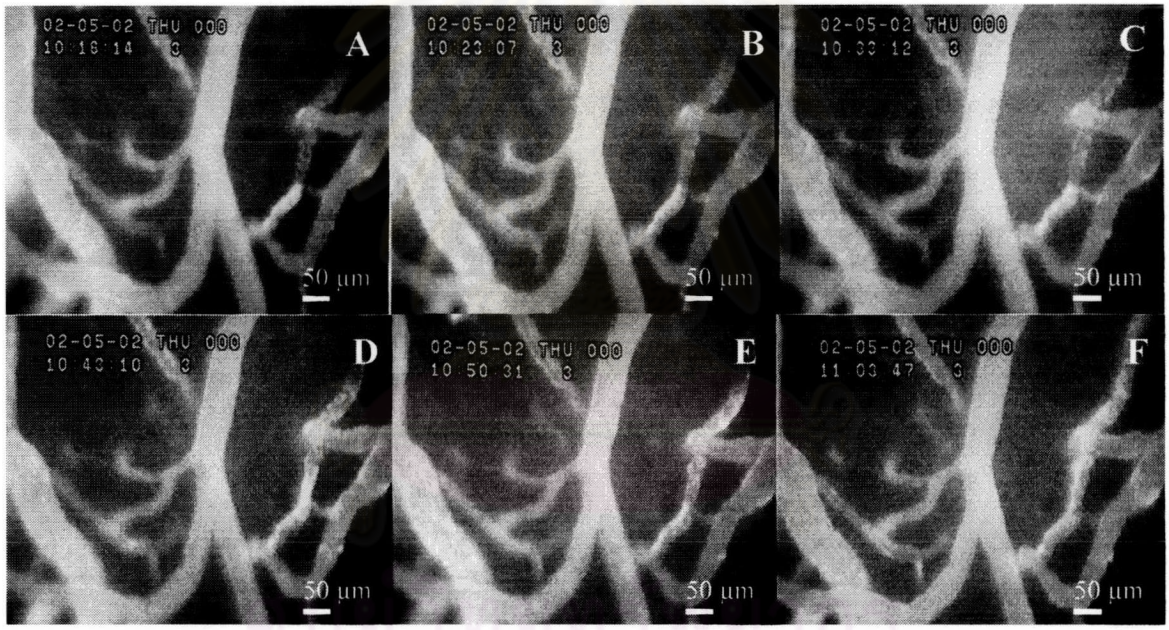

Figure 4.28 *The intravital videomicroscope image of pial arterioles at the maximum vasodilation in each peak in the KCl application with naratriptan-treated group. A, B, C, D, E and F showing at the 1st, 2nd, 3rd, 4th, 5th and 6th peak, respectively. Bar=50 µm.*

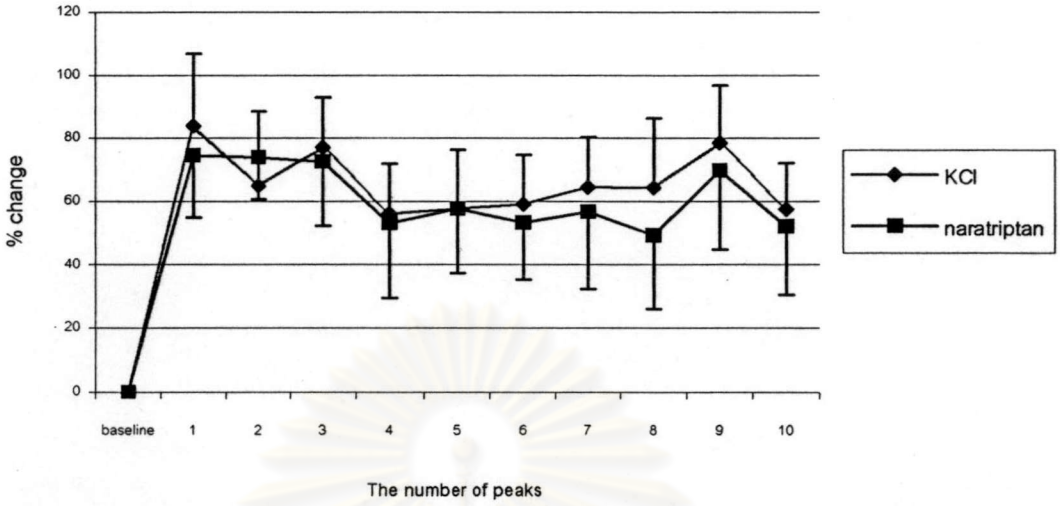

Figure 4.29 *The percent change of pial arteriolar diameter from baseline in KCl application rats with and without naratriptan treatment. No significant difference between two groups was observed. (p>0.05, ANOVA for repeated measurement)*

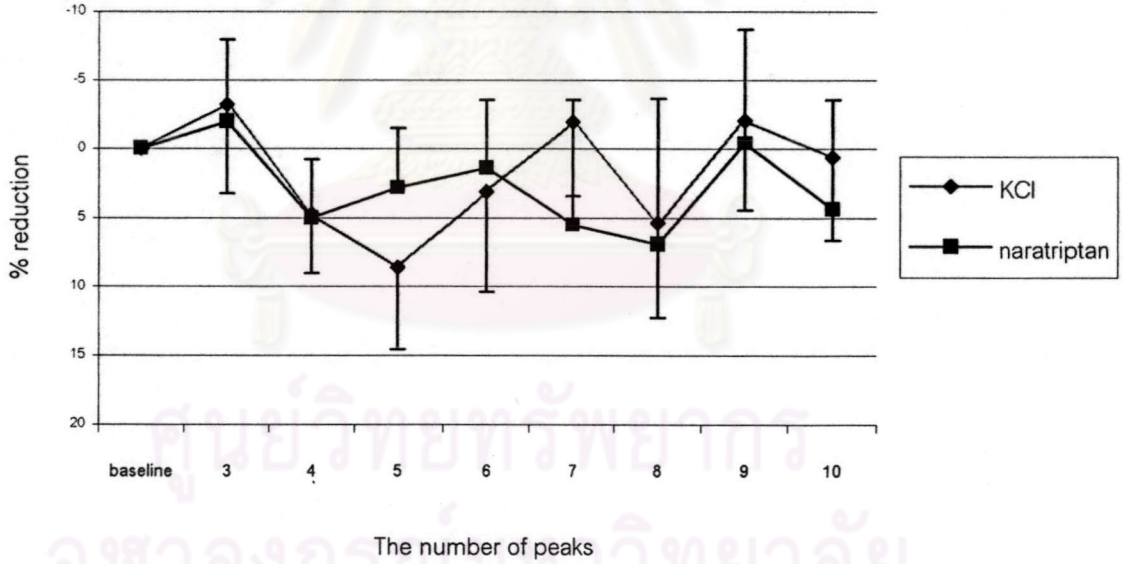

Figure 4.30 *The percent reduction of pial arteriolar diameter from baseline in KCl application rats with and without naratriptan treatment. No significant difference between two groups was observed. (p>0.05, ANOVA for repeated measurement)*