CHAPTER V
RESULTS

The result were divided into 8 parts on these following topics:-

1. The effect of NTG on pial arterioles (diameter about 10-30 μm) of normal rats.

2. The effect of NTG on pial arterioles (diameter about 30-60 μm) of normal rats.

3. The effect of NTG on endothelial cells ultrastructural changes of cerebral capillaries and arterioles of normal rats.

4. The effect of NTG evoked Fos immunoreactivity in brainstem, epithalamus and hypothalamus of normal rats.

5. The effect of NTG on pial arterioles (diameter about 10-30 μm) of PCPA-treated rats compared to saline-treated rats.

6. The effect of NTG on pial arterioles (diameter about 30-60 μm) of PCPA-treated rats compared to saline-treated rats.

7. The effect of NTG on endothelial ultrastructural changes of cerebral capillaries and arterioles of PCPA-treated rats compared to saline-treated rats.

8. The effect of NTG evoked Fos immunoreactivity in brainstem, epithalamus and hypothalamus of PCPA-treated rats compared to saline-treated rats.

Physiological Parameter

Infusion of NTG 10 mg/kg BW within 5 minutes produced a transient decrease in systemic blood pressure. The mean arterial pressure at minute control (0), 5, 15, 30 and 60 were 108.3 ± 10.4, 96.0 ± 4.9, 101.7 ± 4.7, 103.3 ± 4.7 and 103.3 ± 4.7 mmHg, respectively. Mean arterial pressure difference between minute 0 and minute 5 was 12.3 ± 7.2 mmHg (95% CI = 3.4 to 21.3, P=0.019) (Figure 5.1). The blood pressure rapidly became normalized after NTG was removed. NO change in arterial pH, PaO_2 and $PaCO_2$ were evident after NTG infusion. Pretreatment with PCPA did not produce any significant difference in NTG-induced change in systemic arterial pressure compared to those observed in NTG infusion group alone.

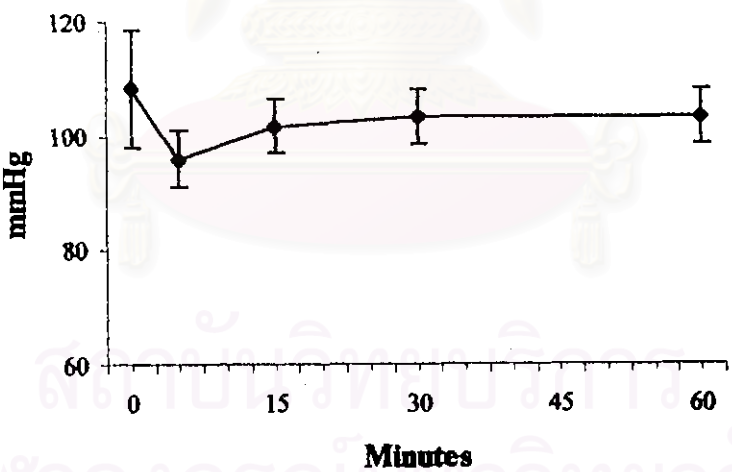


Figure 5.1. Mean arterial blood pressure after NTG 10 mg/kg BW infusion

1. The effect of NTG on pial arterioles (diameter about 10-30 μm) of normal rats.

The videotape recorded from this experiment was played back frame by frame and 6 pial arterioles were randomly selected from each rat (n=5). Images of 30 selected vessels were digitized and the diameters of these vessels were determined as described in chapter IV. The diameters of these arterioles before and after NTG (8 and 10 mg/kg BW) infusion were demonstrated in Table 5.1 and Table 5.2, respectively. The per cent changes from baseline diameter of both groups were shown in Table 5.3 and Table 5.4, respectively.

The per cent changes from baseline diameter of arteriole diameter-time profiles of NTG (8 or 10 mg/kg BW)-treated rats are illustrated in Figure 5.2. The significant pial arteriole dilatation was observed almost immediately after both concentration of NTG infusion and reached its peak at 15 minutes. This effect persisted for at least 60 minutes (Figure 5.3). The greatest difference was evidence at 15 minutes post-infusion. The magnitude of difference at 15 minutes in rat receiving 8 mg/kg BW of NTG = 23.42 $\pm$ 2.73 per cent (95% CI = 18.22 to 28.62, $P < 0.001$) and rat receiving 10 mg/kg BW of NTG = 57.97 $\pm$ 6.39 per cent (95% CI = 44.91 to 71.03, $P < 0.001$). After 30 minutes, the degree of vasodilatation of NTG (8 mg/kg BW) –treated rats began to decline. A higher dose of NTG produced a greater degree of arteriole dilatation. At minutes 15, 30 and 60 post infusion, the per cent dilatation of pial arteriole was statistically greater in the rats receiving 10 mg/kg BW of NTG, compared to those receiving 8 mg/kg BW ($P < 0.001$, ANOVA for repeated measurement with posthoc Bonferroni test) (Figure 5.2).

Table 5.1. The effect of nitroglycerin on rat pial arterioles (diameter about 10-30 μm) before and after 8 mg/kg BW of NTG infusion at 5, 15, 30 and 60 minutes.

Vessels Number	0 min	Diameter after NTG infusion (μm)			
		5 min	15 min	30 min	60 min
1	16	20	20	22	20
2	29	33	32	30	30
3	29	34	34	32	32
4	23	25	27	25	25
5	14	18	16	15	15
6	23	29	28	25	25
7	28	33	33	36	34
8	27	30	30	31	32
9	17	25	25	18	18
10	21	27	28	29	26
11	20	27	26	26	26
12	20	20	23	24	24
13	24	30	36	38	36
14	21	24	24	24	23
15	25	29	26	26	26
16	21	28	29	31	31
17	25	32	30	30	30
18	22	25	25	25	23
19	26	29	32	32	32
20	25	30	28	28	28
21	12	26	16	16	15
22	21	22	26	28	26
23	16	26	27	23	23
24	25	26	28	30	28
25	30	33	33	33	32
26	17	21	21	22	24
27	16	18	22	20	20
28	14	17	17	15	15
29	21	25	25	22	22
30	17	17	20	20	20
Average	21.50	25.97*	26.23*	25.87*	25.37*
S.E.	0.90	0.92	0.96	1.09	1.04

*$P < 0.001$ compared to control

Table 5.2. The effect of nitroglycerin on rat pial arterioles (diameter about 10-30 μm) before and after 10 mg/kg BW of NTG infusion at 5, 15, 30 and 60 minutes.

Vessels Number	0 min	Diameter after NTG infusion (μm)			
		5 min	15 min	30 min	60 min
1	17	20	26	25	25
2	22	30	33	33	39
3	25	33	33	30	30
4	14	24	31	20	20
5	20	37	39	35	32
6	15	22	22	20	20
7	18	18	20	20	22
8	16	17	21	17	16
9	25	28	33	33	35
10	30	35	55	56	55
11	25	40	50	60	55
12	30	40	60	60	55
13	16	21	23	22	22
14	22	30	33	33	34
15	15	23	24	19	18
16	13	24	30	30	27
17	25	32	42	54	46
18	30	32	39	34	30
19	12	15	16	19	15
20	15	15	20	26	24
21	18	25	28	35	28
22	15	28	37	33	30
23	12	12	15	14	13
24	18	24	26	29	23
25	19	34	34	20	20
26	13	14	15	18	18
27	11	12	17	18	18
28	11	16	18	21	17
29	21	22	28	25	23
30	30	35	35	36	35
Average	19.10	25.27*	30.10*	29.83*	28.17*
S.E.	1.11	1.52	2.09	2.34	2.17

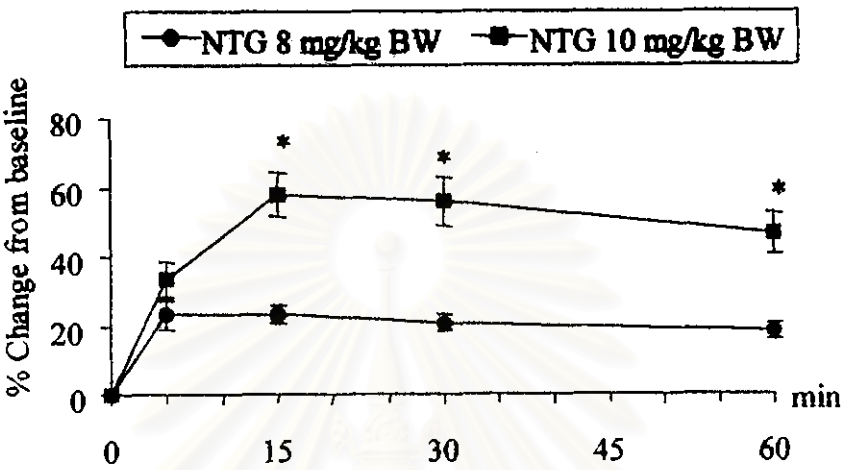
*$P < 0.001$ compared to control

Table 5.3. The per cent change from baseline diameter of rat pial arterioles (diameter about 10-30 μm) after 8 mg/kg BW of NTG infusion at 5, 15, 30 and 60 minutes.

Vessels	% Changes after NTG infusion			
Number	5 min	15 min	30 min	60 min
1	25.00	25.00	37.50	25.00
2	13.79	10.34	3.45	3.45
3	17.24	17.24	10.34	10.34
4	8.70	17.39	8.70	8.70
5	28.57	14.29	7.14	7.14
6	26.09	21.74	8.70	8.70
7	17.86	17.86	28.57	21.43
8	11.11	11.11	14.81	18.52
9	47.06	47.06	5.88	5.88
10	28.57	33.33	38.10	23.81
11	35.00	30.00	30.00	30.00
12	0.00	15.00	20.00	20.00
13	25.00	50.00	58.33	50.00
14	14.29	14.29	14.29	9.52
15	16.00	4.00	4.00	4.00
16	33.33	38.10	47.62	47.62
17	28.00	20.00	20.00	20.00
18	13.64	13.64	13.64	4.55
19	11.54	23.08	23.08	23.08
20	20.00	12.00	12.00	12.00
21	16.67	33.33	33.33	25.00
22	4.76	23.81	33.33	23.81
23	62.50	68.75	43.75	43.75
24	4.00	12.00	20.00	12.00
25	10.00	10.00	10.00	6.67
26	23.53	23.53	29.41	41.18
27	12.50	37.50	25.00	25.00
28	21.43	21.43	7.14	7.14
29	19.05	19.05	4.76	4.76
30	0.00	17.65	17.65	17.65
Average	23.17	23.42	21.02	18.69
S.E.	4.03	2.54	2.62	2.43

Table 5.4. The per cent change from baseline diameter of rat pial arterioles (diameter about 10-30 μm) after 10 mg/kg BW of NTG infusion at 5, 15, 30 and 60 minutes.

Vessels Number	% Changes after NTG infusion			
	5 min	15 min	30 min	60 min
1	17.65	52.94	47.06	47.06
2	36.36	50.00	50.00	77.27
3	32.00	32.00	20.00	20.00
4	71.43	121.43	42.86	42.86
5	85.00	95.00	75.00	60.00
6	46.67	46.67	33.33	33.33
7	0.00	11.11	11.11	22.22
8	6.25	31.25	6.25	0.00
9	12.00	32.00	32.00	40.00
10	16.67	83.33	86.67	83.33
11	60.00	100.00	140.00	120.00
12	33.33	100.00	100.00	83.33
13	31.25	43.75	37.50	37.50
14	36.36	50.00	50.00	54.55
15	53.33	60.00	26.67	20.00
16	84.62	130.77	130.77	107.69
17	28.00	68.00	116.00	84.00
18	6.67	30.00	13.33	0.00
19	25.00	33.33	58.33	25.00
20	0.00	33.33	73.33	60.00
21	38.89	55.56	94.44	55.56
22	86.67	146.67	120.00	100.00
23	0.00	25.00	16.67	8.33
24	33.33	44.44	61.11	27.78
25	78.95	78.95	5.26	5.26
26	7.69	15.38	38.46	38.46
27	9.09	54.55	63.64	63.64
28	45.45	63.64	90.91	54.55
29	4.76	33.33	19.05	9.52
30	16.67	16.67	20.00	16.67
Average	33.47	57.97	55.99	46.60
S.E.	4.96	6.39	7.11	5.96



* $P < 0.001$ compared to NTG 8 mg/kg BW infusion

Figure 5.2. The per cent change from baseline of rat pial microvessel diameter (% from baseline) in cerebral arteriole (diameter about 10-30 μm) after NTG (8 and 10 mg/kg BW) infusion at 5, 15, 30 and 60 minutes.

Figure 5.3. Intravital videomicroscope image of pial microvessels before and after infusion of NTG 10 mg/kg BW in 5 minutes.

A) Before infusion
B) 15 min after infusion
C) 30 min after infusion
D) 60 min after infusion

Significant dilatation of pial microvessels is demonstrated. Bar = 50 μm.

2. The effect of NTG on pial arterioles (diameter about 30-60 μm) of normal rats.

The videotape recorded from this experiment was played back frame by frame and 3 pial arterioles were randomly selected from each rat (n=5). Images of 15 selected vessels were digitized and the diameters of these vessels were determined as described in chapter IV. The diameters of these arterioles before and after NTG (8 and 10 mg/kg BW) infusion were demonstrated in Table 5.5 and Table 5.6, respectively. The per cent changes from baseline diameter of both groups were shown in Table 5.7 and Table 5.8, respectively.

The per cent changes from baseline of arteriole diameter-time profiles of NTG (8 or 10 mg/kg BW)-treated rats are illustrated in Figure 5.4. The pial arteriole dilatation was observed almost immediately after both concentration of NTG infusion and reached its peak at 30 minutes. This effect persisted for at least 60 minutes. The greatest difference was evidence at 30 minutes post-infusion. The magnitude of difference at 30 minutes in rat receiving 8 mg/kg BW of NTG = 13.11 $\pm$ 1.92 per cent (95% CI = 9.00 to 17.22, $P < 0.001$) and rat receiving 10 mg/kg BW of NTG = 36.72 $\pm$ 6.46 per cent (95% CI = 22.86 to 50.58, $P < 0.001$). After 30 minutes, the degree of vasodilatation of NTG (8 mg/kg BW)-treated rats began to decline. A higher dose of NTG produced a greater degree of arteriole dilatation. At minutes 15, 30 and 60 post infusion, the per cent dilatation of pial arteriole was statistically greater in the rats receiving 10 mg/kg BW of NTG, compared to those receiving 8 mg/kg BW ($P <$ 0.001, ANOVA for repeated measurement with posthoc Bonferroni test) (Figure 5.4).

Table 5.5. The effect of nitroglycerin on rat pial arterioles (diameter about 30-60 μm) before and after 8 mg/kg BW of NTG infusion at 5, 15, 30 and 60 minutes.

Vessels Number	0 min	Diameter after NTG infusion (μm)			
		5 min	15 min	30 min	60 min
1	40	44	48	50	49
2	36	38	42	44	44
3	32	40	35	35	32
4	57	57	65	64	57
5	54	64	62	60	57
6	50	57	54	54	52
7	42	54	50	50	50
8	60	61	64	60	60
9	35	38	38	38	38
10	56	56	59	59	58
11	31	37	37	37	39
12	31	32	33	33	33
13	56	56	60	61	61
14	32	32	36	38	38
15	45	46	52	55	55
Average	43.80	47.47*	49.00*	49.20*	48.20*
S.E.	2.79	2.81	2.96	2.79	2.60

*$P < 0.001$ compared to control

Table 5.6. The effect of nitroglycerin on rat pial arterioles (diameter about 30-60 μm) before and after 10 mg/kg BW of NTG infusion at 5, 15, 30 and 60 minutes.

Vessels Number	0 min	Diameter after NTG infusion (μm)			
		5 min	15 min	30 min	60 min
1	45	47	70	69	60
2	39	60	60	67	60
3	50	65	65	68	75
4	32	44	44	46	42
5	51	51	55	56	55
6	50	55	55	52	52
7	35	40	40	40	42
8	51	54	64	62	60
9	40	56	60	64	65
10	51	67	79	78	78
11	32	52	55	60	60
12	34	34	43	46	49
13	40	40	43	43	41
14	40	56	60	55	54
15	58	66	68	67	66
Average	43.20	52.47*	57.40*	58.20*	57.27*
S.E.	2.13	2.58	2.91	2.87	2.89

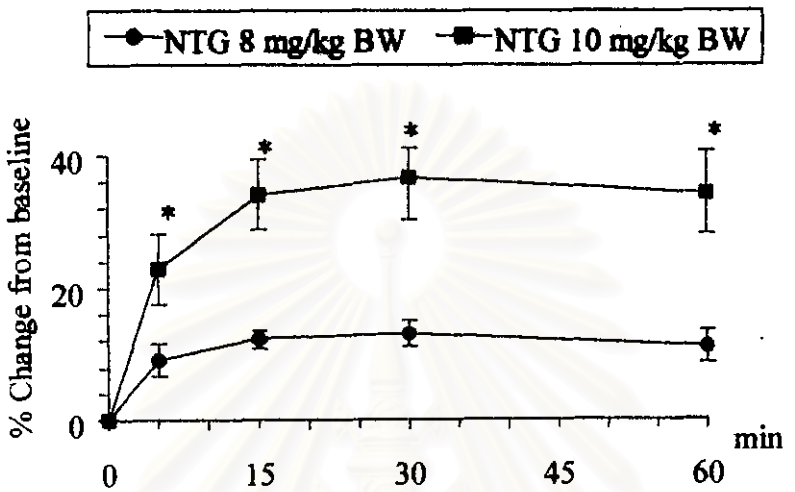
*$P < 0.001$ compared to control

Table 5.7. The per cent change from baseline diameter of rat pial arterioles (diameter about 30-60 μm) after 8 mg/kg BW of NTG infusion at 5, 15, 30 and 60 minutes.

Vessels Number	% Changes after NTG infusion			
	5 min	15 min	30 min	60 min
1	10.00	20.00	25.00	22.50
2	5.56	16.67	22.22	22.22
3	25.00	9.38	9.38	0.00
4	0.00	14.04	12.28	0.00
5	18.52	14.81	11.11	5.56
6	14.00	8.00	8.00	4.00
7	28.57	19.05	19.05	19.05
8	1.67	6.67	0.00	0.00
9	8.57	8.57	8.57	8.57
10	0.00	5.36	5.36	3.57
11	19.35	19.35	19.35	25.81
12	3.23	6.45	6.45	6.45
13	0.00	7.14	8.93	8.93
14	0.00	12.50	18.75	18.75
15	2.22	15.56	22.22	22.22
Average	9.11	12.24	13.11	11.18
S.E.	2.52	1.34	1.92	2.45

Table 5.8. The per cent change of rat pial arterioles (diameter about 30-60 μm) after 10 mg/kg BW of NTG infusion at 5, 15, 30 and 60 minutes.

Vessels	% Changes after NTG infusion			
Number	5 min	15 min	30 min	60 min
1	4.44	55.56	53.33	33.33
2	53.85	53.85	71.79	53.85
3	30.00	30.00	36.00	50.00
4	37.50	37.50	43.75	31.25
5	0.00	7.84	9.80	7.84
6	10.00	10.00	4.00	4.00
7	14.29	14.29	14.29	20.00
8	5.88	25.49	21.57	17.65
9	40.00	50.00	60.00	62.50
10	31.37	54.90	52.94	52.94
11	62.50	71.88	87.50	87.50
12	0.00	26.47	35.29	44.12
13	0.00	7.50	7.50	2.50
14	40.00	50.00	37.50	35.00
15	13.79	17.24	15.52	13.79
Average	22.91	34.17	36.72	34.42
S.E.	5.33	5.36	6.46	6.26



* $P < 0.001$ compared to NTG 8 mg/kg BW infusion

Figure 5.4. The per cent change from baseline of rat pial microvessel diameter (% from baseline) in cerebral arteriole (diameter about 30-60 μm) after NTG (8 and 10 mg/kg BW) infusion at 5, 15, 30 and 60 minutes.

3. The effect of NTG on endothelial cells ultrastructural changes in of cerebral capillaries and arterioles of normal rats.

Criteria of Morphometric Study

Morphological changes in the ultrastructure of endothelial cells of cerebral microvessels were quantified. To define possible differences between ultrastructural changes in capillaries and arterioles, the studied pial microvessels were divided into two groups based on their diameter. Capillaries were defined as such if their diameters were equal or less than 10 μm. Vessels with a diameter about 15-30 μm were defined as arterioles. The Morphometric parameters studied included the number of microvilli, number of intracytoplasmic pinocytic vesicles, mitochondrial diameter and blood vessel separation from adjacent tissue due to the astrocytic foot-plate swollen.

The number of microvilli was determined in 5 control, 5 NTG-treated rats. The numbers of endothelium microvilli were randomly counted from 10 vessels of diameter about 15-30 μm and 20 vessels of diameter less than 10 μm from each rat and were expressed as number of microvilli per vessel.

The densities of pinocytic vesicles were investigated in 5 controls, 5 NTG rats. The densities of pinocytic vesicles from each rat were quantified by counting their numbers in 5 randomly selected micrographs (x34,000) taken from each vessel. Regarding the area selection, a transparent sheet with a 6.7 mm square grid was fixed to the micrographs. In each case, at least 25 squares of random area available for quantitative analysis. (1 square grid represent 0.04 μm^2, therefore 25 blocks represent

1 μm^2 in real scale). The obtained numbers were averaged and expressed as number per square micrometer.

The diameter of mitochondria of cerebral endothelial cell were defined by randomly measured from the micrograph (x51,000) taken from 10 vessels of diameter about 15-30 μm and 20 vessels of diameter less than 10 μm from each rat. As the average of diameter of endothelial mitochondria in control vessels diameter about 15-30 μm was 167.42 ± 38.67 nm (average over 95 control mitochondria) and 154.76 ± 39.95 nm (average over 93 control mitochondria) in control vessel diameter less than 10 μm. Mitochondria with diameter greater than mean + 2SD of normal length were defined as swollen and were counted. From these criteria, mitochondria with a diameter above 245 nm in blood vessel diameter about 15-30 μm and 235 nm in blood vessel diameter less than 10 μm were defined as being swollen and counted. The same morphometry was applied to every groups.

Endothelium separation from brain tissue was randomly observed and counted from 10 vessels of diameter about 15-30 μm and 20 vessels of diameter less than 10 μm from 5 control and 5 NTG-rats.

Results

Microvillous Formation. A significant increase in the number of endothelial microvilli was evident in NTG-treated group compared with the control (Table 5.9, Figure 5.5). The average number of microvilli in capillaries were 0.05 ± 0.35, 1.37 ± 0.05 and 2.83 ± 1.66 microvilli per vessel for the control, NTG (8mg/kg BW) and NTG (10 mg/kg BW)–treated group, respectively. The significant increase of the number of endothelial microvilli was observed in arterioles. The average number of

microvilli in these arterioles were 1.28 ± 0.86, 3.4 ± 1.74 and 9.16 ± 3.54 for the control, NTG (8mg/kg BW) and NTG (10mg/kg BW)–treated group, respectively ($P < 0.05$) (Figure 5.6).

Number of Pinocytic Vesicle. Only a low number of pinocytic vesicles was observed in endothelial cells of the cerebral microvessels in the control and NTG (8 mg/kg BW) –treated group. These vesicles became more abundant in endothelial cell of cerebral microvessels taken from NTG (10 mg/kg BW)-treated rats. (Table 5.10) Increased pinocytosis was demonstrated on both the luminal and abluminal surface of cerebral microvessels (Figure 5.8). The average density of pinocytic vesicles in cerebral capillaries was 9.92 ± 2.75, 8.56 ± 3.74 and 27.28 ± 2.76 vesicles/μm^2, for the control, NTG (8 mg/kg BW) and NTG (10 mg/kg BW)-treated groups, respectively. In arterioles, the density of pinocytic vesicles was 9.68 ± 1.56, 9.86 ± 2.55 and 27.84 ± 3.49 vesicles/ μm^2, for the control, NTG (8 mg/kg BW) and NTG (10 mg/kg BW)-treated rats respectively. The number of pinocytic vesicle in endothelial cells of cerebral microvessels were significantly increased in NTG (10 mg/kg BW)- treated rat ($P < 0.05$) (Figure 5.7).

Mitochondrial Diameter. The average mitochondrial diameter in cerebral capillaries and arterioles taken from the control group was 154.76 ± 39.95 and 167.42 ± 38.67 nm, respectively. A significant increase in mitochondria diameter was evident in NTG-treated group ($P < 0.05$). (Table 5.11 and Figure 5.8) The average mitochondrial diameter of the NTG (10 mg/kg BW)-treated rats was 210.61 ± 8.55 and 221.59 ± 25.75 nm for capillaries and arterioles, respectively. The percentage of mitochondria with diameter greater than mean + 2SD of normal length (235 nm in capillary and 245 nm in arteriole) was also greater in the NTG (10 mg/kg BW)-treated group (31.18 % and 4.5 % for capillaries of NTG-

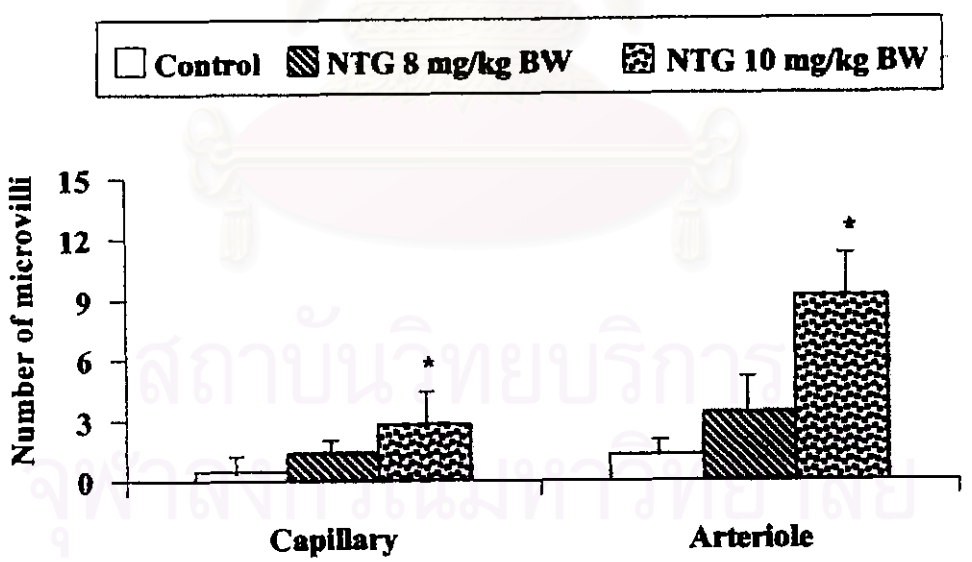
treated and control groups, respectively, odd ratio 9.63, 95 % CI = 3.01 to 34.13, $P < 0.001$ and 23.53 % and 3.16 % for arterioles of NTG-treated and control groups, respectively, odd ratio 9.78, 95 % CI= 4.11 to 23.91, $P < 0.01$ (Table 5.12).

Other Morphological Changes. Possible abnormal features observed in the NTG-treated groups included swelling of perivascular astrocytic foot plate, separation of endothelial cell from its adjacent tissues and dome-like protrusion of endothelial cells into the luminal surface were demonstrated in Figure 5.9 and 5.10. These features were not evidenced in the control group. The number of capillaries with perivascular astrocytic foot plate swelling and/or partial basement membrane separation observed in 100 vessels in the control and NTG (10 mg/kg BW)-treated groups was 2 and 29 vessels, respectively (odd ratio = 22.15, 95 % CI = 4.50 to 47.64, $P< 0.001$). In arterioles, the number of vessels with partial membrane separation observed in 50 vessels were 2 and 24 vessels for the control and NTG (10 mg/kg BW)-treated group respectively (odd ratio 20.01, 95 % CI = 4.43 to 125.54, $P < 0.001$) (Table 5.13). No significant change in nuclei of endothelial cells was noted. No significant structural change was evidenced in the smooth muscle cell layer.

Table 5.9. The mean±SD of the number of microvilli/vessel in the endothelial cell of cerebral capillaries (diameter range from 5-10 μm) (n=100) and arterioles (diameter range from 15-30 μm) (n=50) obtained from control and NTG-treated rats.

Group	Microvilli (number/vessel)	
	Capillary	Arteriole
Control	0.50 ± 0.35	1.28 ± 0.86
NTG 8 mg/kg BW	1.37 ± 0.50	3.40 ± 1.74
NTG 10 mg/kg BW	2.83 ± 1.66*	9.16 ± 3.54*

* $P < 0.05$ compared to control



* $P < 0.05$ compared to control

Figure. 5.5 Bar graph showing the mean±SD of the number of microvilli/vessel in the endothelial cell of cerebral capillaries (diameter range from 5-10 μm) (n=100) and arterioles (diameter range from 15-30 μm) (n=50) obtained from control and NTG-treated rats.

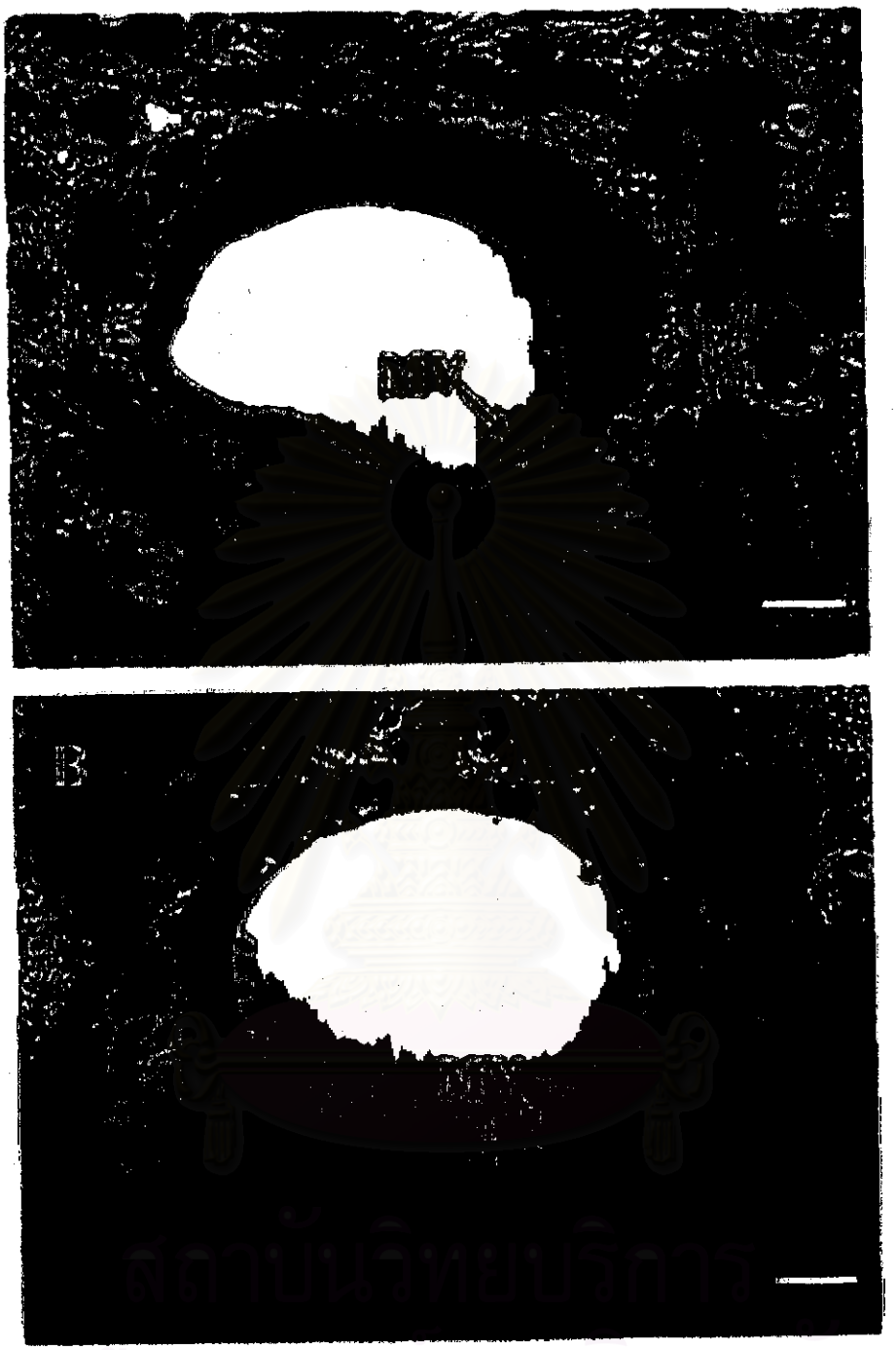
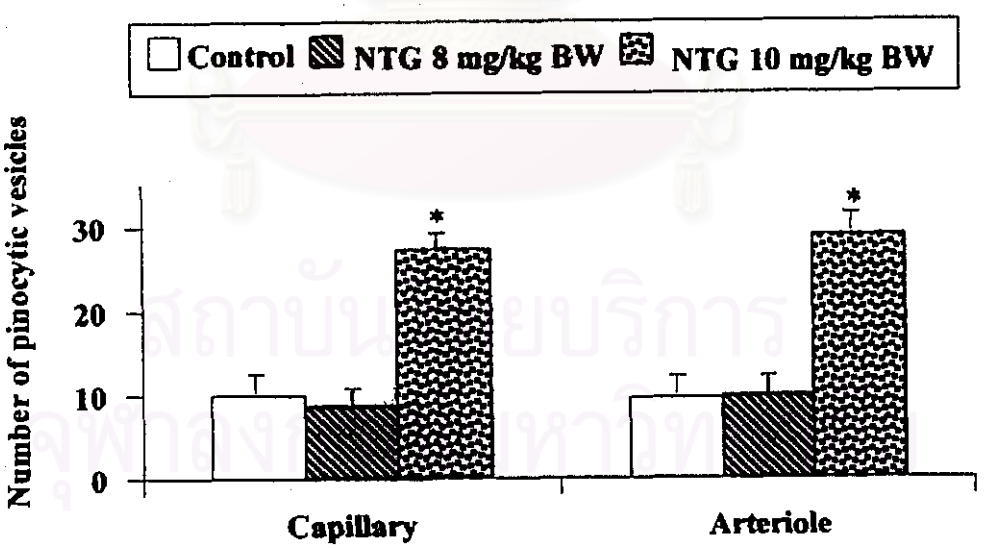


Figure 5.6 Electron micrograph of cerebral microvessels from (A) control and (B) NTG (10 mg/kg BW)-treated rats showing an increase in microvillous formation in cerebral capillaries. (x6000) Bar = 1 μm, MV=microvilli.

Table 5.10. The mean±SD of the number of pinocytic vesicle/μm^2 in the endothelial cell of cerebral capillaries (diameter range from 5-10 μm) and arterioles (diameter range from 15-30 μm) (n=25) obtained from control and NTG-treated rats.

Group	pinocytic vesicle(number/μm^2)	
	Capillary	Arteriole
Control	9.92 ± 2.75	9.68 ± 1.56
NTG 8 mg/kg BW	8.56 ± 3.74	9.86 ± 2.55
NTG 10 mg/kg BW	27.28 ± 2.76*	28.84 ± 3.49*

* $P < 0.05$ compared to control



* $P < 0.05$ compared to control

Figure 5.7 Bar graph showing the mean±SD of the number of pinocytic vesicle/μm^2 in the endothelial cell of cerebral capillaries (diameter range from 5-10 μm) and arterioles (diameter range from 15-30 μm) (n=25) obtained from control and NTG-treated rats.

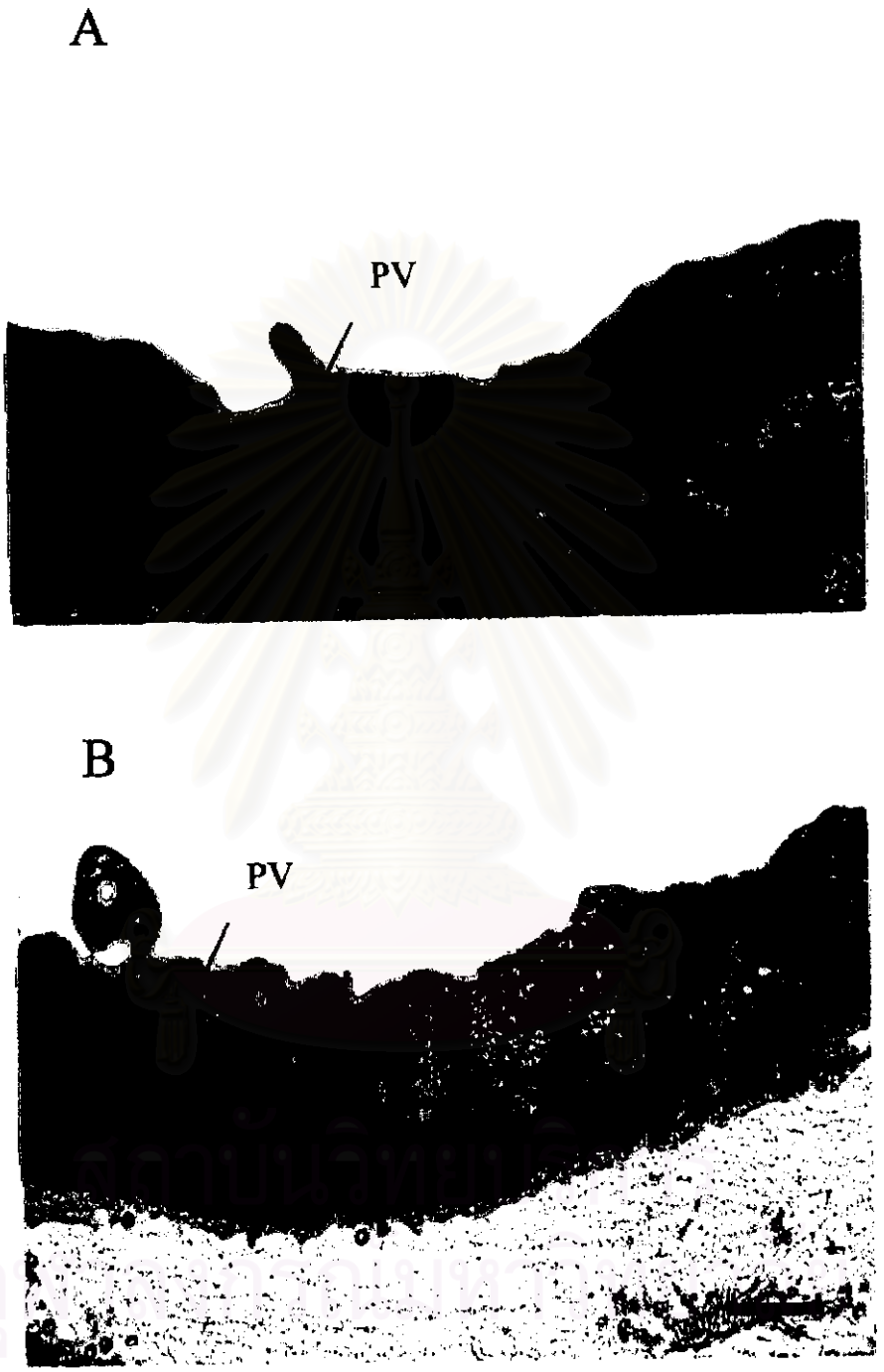


Figure 5.8. Electron micrograph of cerebral microvessels from (A) control and (B) NTG-treated rats showing and increase in density of pinocytic vesicles in endothelial cells. (x34,000). Bar = 200 nm, PV=Pinocytic vesicle.

Table 5.11. The mean±SD of endothelial mitochondrial diameter of capillaries and arterioles obtained from control and NTG (10 mg/kg BW)-treated rats.

Group	Mitochondrial diameter (nm)	
	Capillary	Arteriole
Control	154.76 ± 39.95	167.42 ± 38.67
NTG-treated	210.61 ± 8.55*	221.59 ± 25.75*

* $P < 0.05$ compared to control

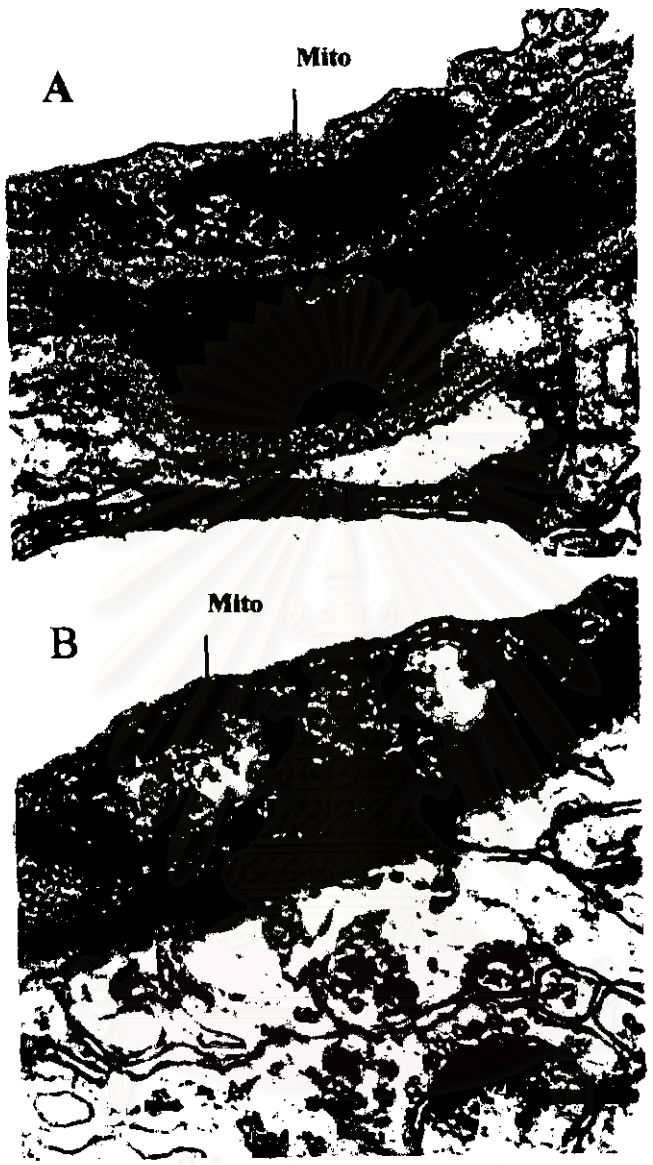


Figure 5.9 Electron micrograph of endothelial cells of cerebral microvessels showing (A) mitochondria of control group and (B) the ballooning of mitochondria with disruption of cristae of NTG (10 mg/kg BW)-treated rats. (x51,000)

Bar = 200 nm , Mito = Mitochondria

Table 5.12. The number mitochondrial changes of control and NTG-treated rats.

a) capillaries

b) arterioles

Significance of difference was determined by chi-square test.

(a)

Number	Mitochondria		Total
	Normal	Swollen	
Control	85	4	89
NTG	64	29	93
Total	149	33	182

Odd ratio = 9.63

95% confidence interval (3.01-34.13)

$P< 0.001$

(b)

Number	Mitochondria		Total
	Normal	Swollen	
Control	92	3	95
NTG	65	20	85
Total	157	23	180

Odd ratio = 9.78

95% confidence interval (4.11-23.91)

$P< 0.001$

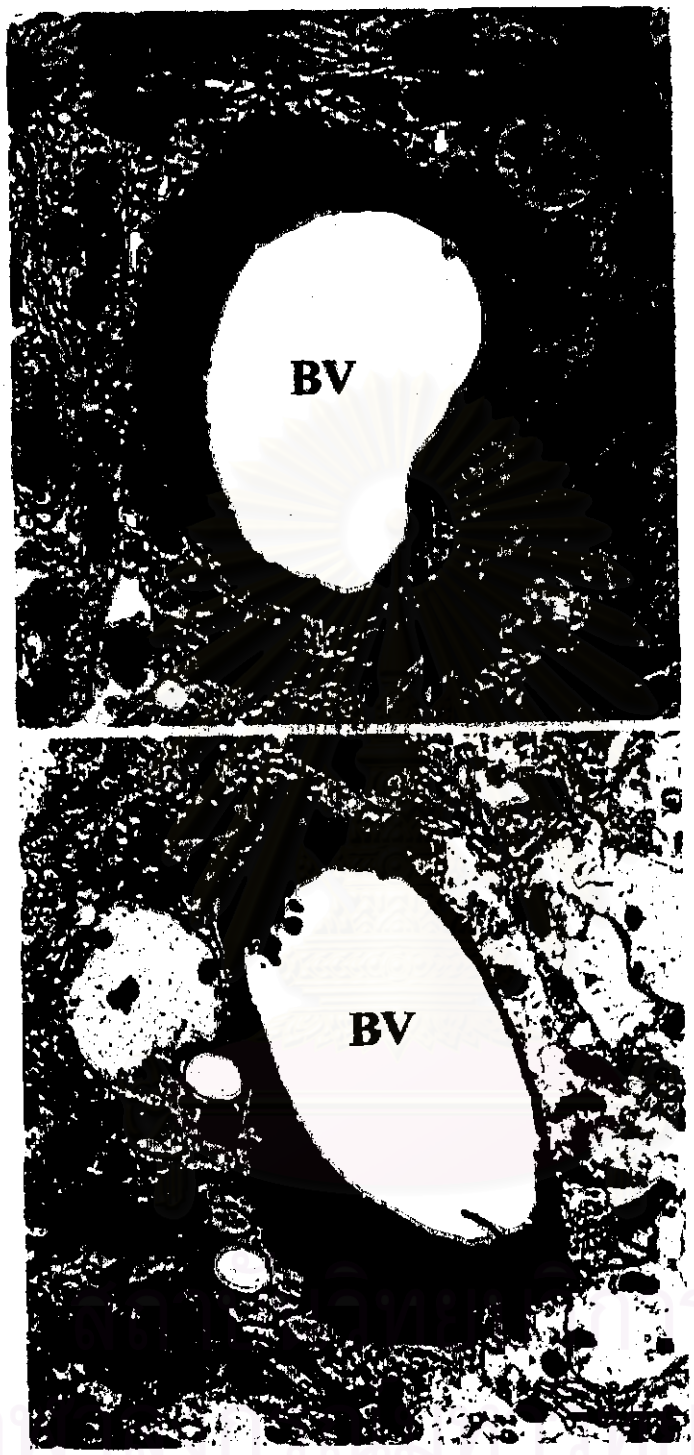


Figure 5.10. Electron micrograph of cerebral microvessels from (A) control and (B) NTG (10 mg/kg BW)-treated rats showing the separation of a cerebral microvessel from adjacent brain tissue. (x6,000)
Bar = 1 μm, BV=blood vessel.

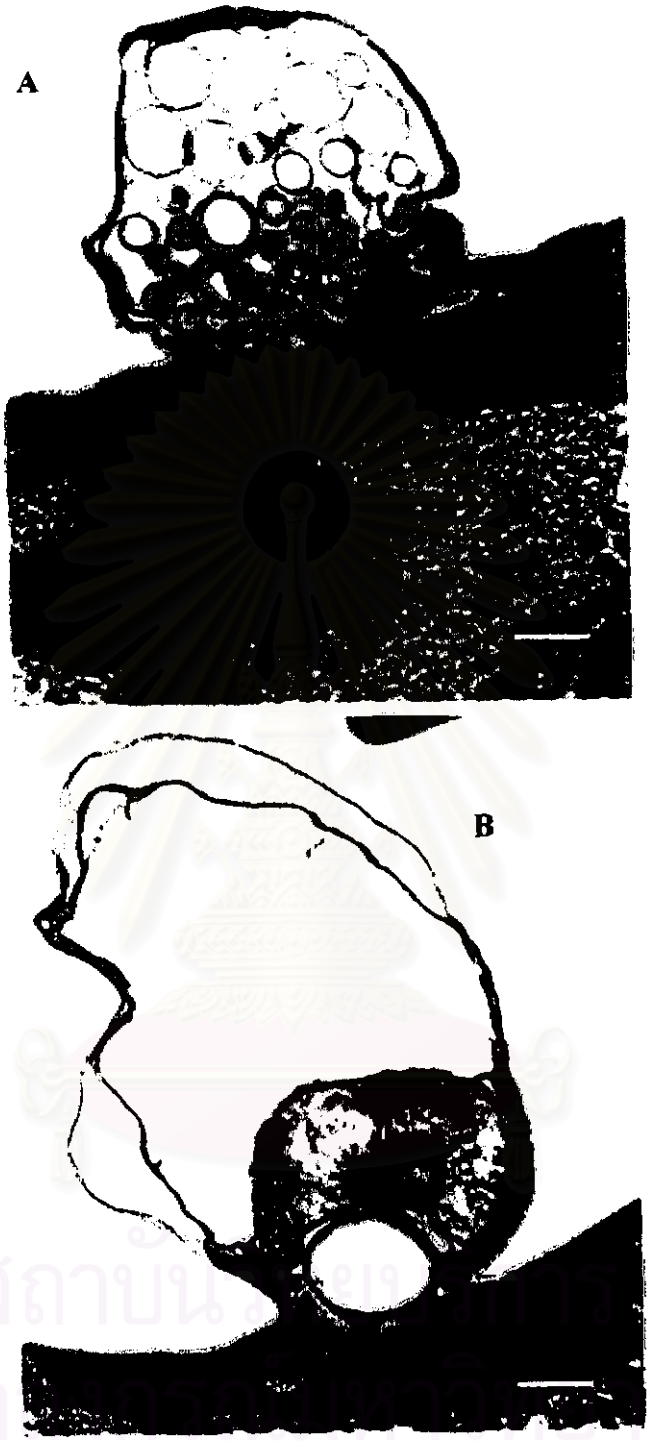


Figure 5.11. Electron micrograph of luminal surface of cerebral endothelial cells Obtained from NTG (10 mg/kg BW)-treated rat showing (A) dome-like protrusion of luminal surface and (B) ballooning of endothelial surface. (x51,000) Bar = 200 nm

Table 5.13. Showing the number of endothelium separation from basement membrane in blood vessel of NTG (10 mg/kgBW)- treated and control rats obtained from

a) capillaries

b) arterioles

The significance of difference was determined by chi-square test

(a)

Number	Endothelial separation		Total
	Normal	Abnormal	
Control	98	2	100
NTG	71	29	100
Total	169	31	200

Odd ratio = 22.15

95% confidence interval (4.50-47.64)

$P < 0.001$

(b)

Number	Endothelial separation		Total
	Normal	Abnormal	
Control	48	2	50
NTG	26	24	50
Total	74	26	100

Odd ratio = 20.01

95% confidence interval (4.43-125.54)

$P < 0.001$

4. The effect of NTG evoked Fos immunoreactivity in brainstem, epithalamus and hypothalamus of normal rats.

As shown in camera lucida drawings of coronal section through the brainstem (Figure 5.12) In sham animals, only scattered immunoreactive neurons were observed in the brainstem segments. In NTG (10 mg/kg BW) infusion group, extensive Fos immunoreactivity were observed in several brainstem nuclei. Numerous positive cells were observed in nucleus tractus solitarius (NTS) bilaterally (Figure 5.13). Moderate number of labelled cells were found bilaterally in lateral recticular nucleus (LRN) (Figure 5.14) and in trigeminal nucleus caudalis TNC (Figure 5.15). Finally, the NTG-treated rats displayed bilateral FOS-like immunoreactivity in the inferior olive (IO) (Figure 5.16).

In epithalamus and hypothalamus, as shown in camera lucida drawings of coronal sections through the epithalamus (Figure 5.17). Fos-like immunoreactivity was found bilaterally and most intensely within cells of the epithalamus such as habenular (Figure 5.18) and in hypothalamus, fos-like immunoreactive cells were found in supraoptic (Figure 5.19) and paraventricular nuclei (Figure 5.20). In contrary, only scattered immunoreactive neurons were observed in the same area of control rats.

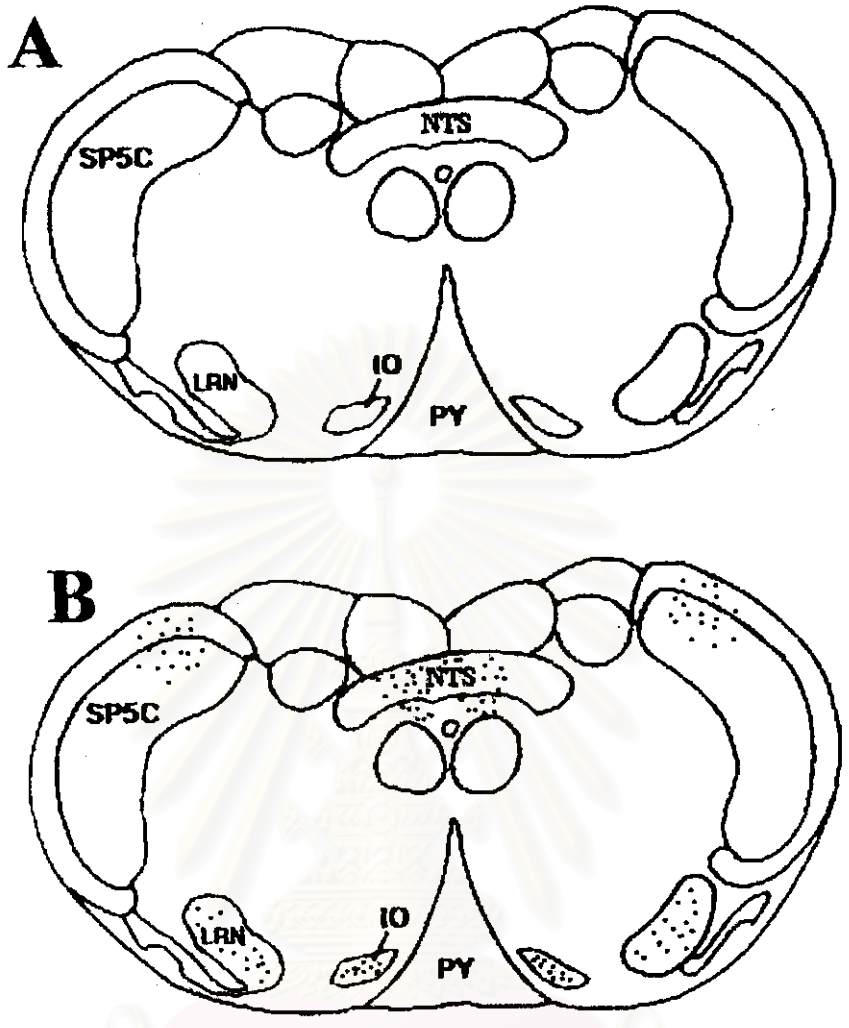


Figure 5.12. The camera lucida drawing of coronal section of the brainstem in A) sham rat and B) NTG-treated rat. (SP5C=spinal trigeminal nucleus caudalis, NTS=nucleus tractus solitarius, LRN=lateral recticular nucleus, IO=inferior olive and PY=pyramidal tract)

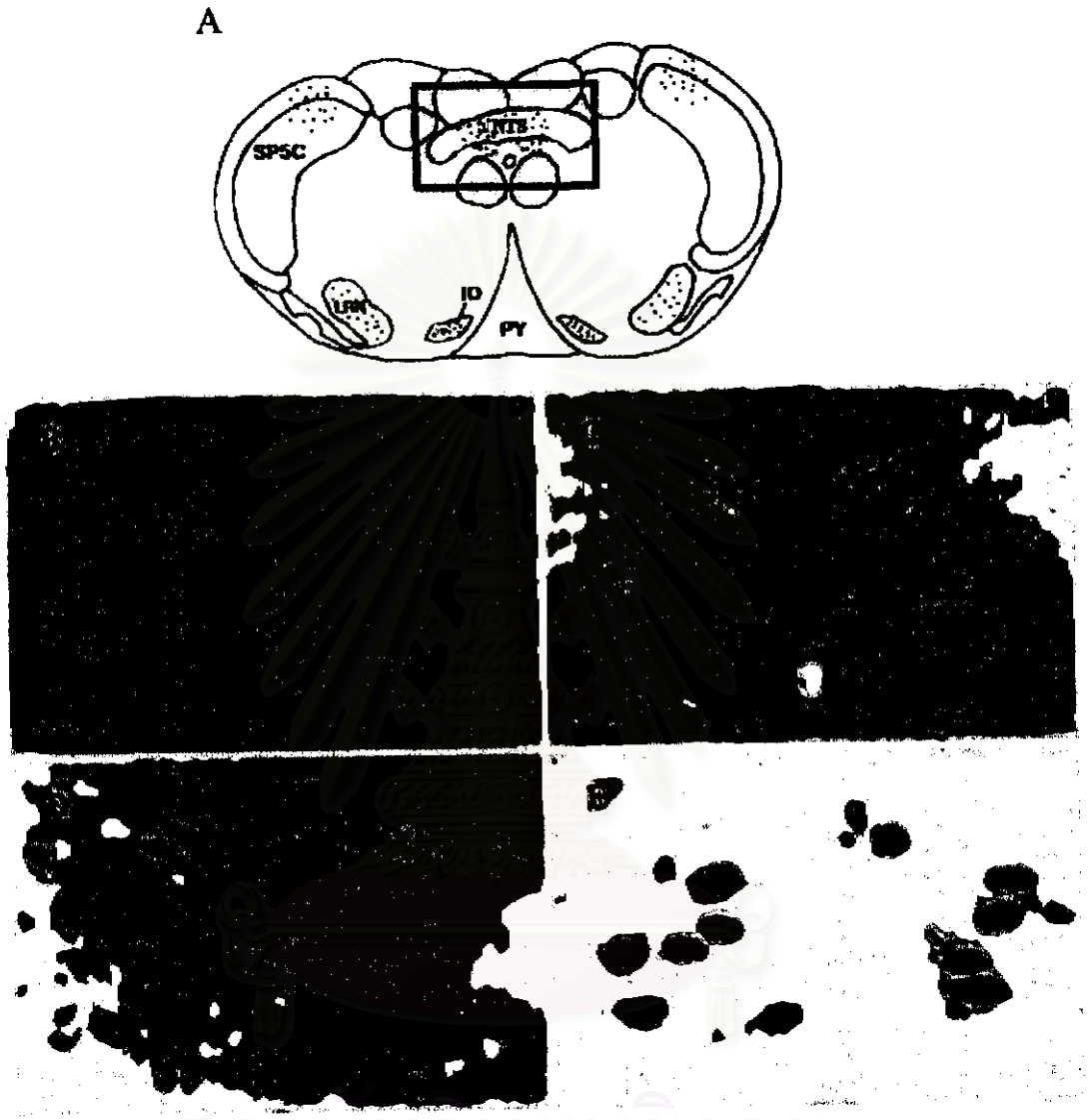


Figure 5.13. Photomicrographs showing the Fos immunoreactivity in NTS of the brainstem of the rat. Fos positive cells (arrow) are easily visible and well differentiated from the background after NTG (10 mg/kg BW) infusion. Box in the schematic drawings indicate the area the respective photographs were taken from. (A) Camera lucida drawing of brainstem of NTG-treated rat, (B) Control (C) NTG-treated rat (x4), (D) higher magnification of (C) (x20) and (E) higher magnification of (C) (x40). (SP5C=spinal trigeminal nucleus caudalis, NTS=nucleus tractus solitarius, LRN=lateral recticular nucleus, IO=inferior olive and PY=pyramidal tract)

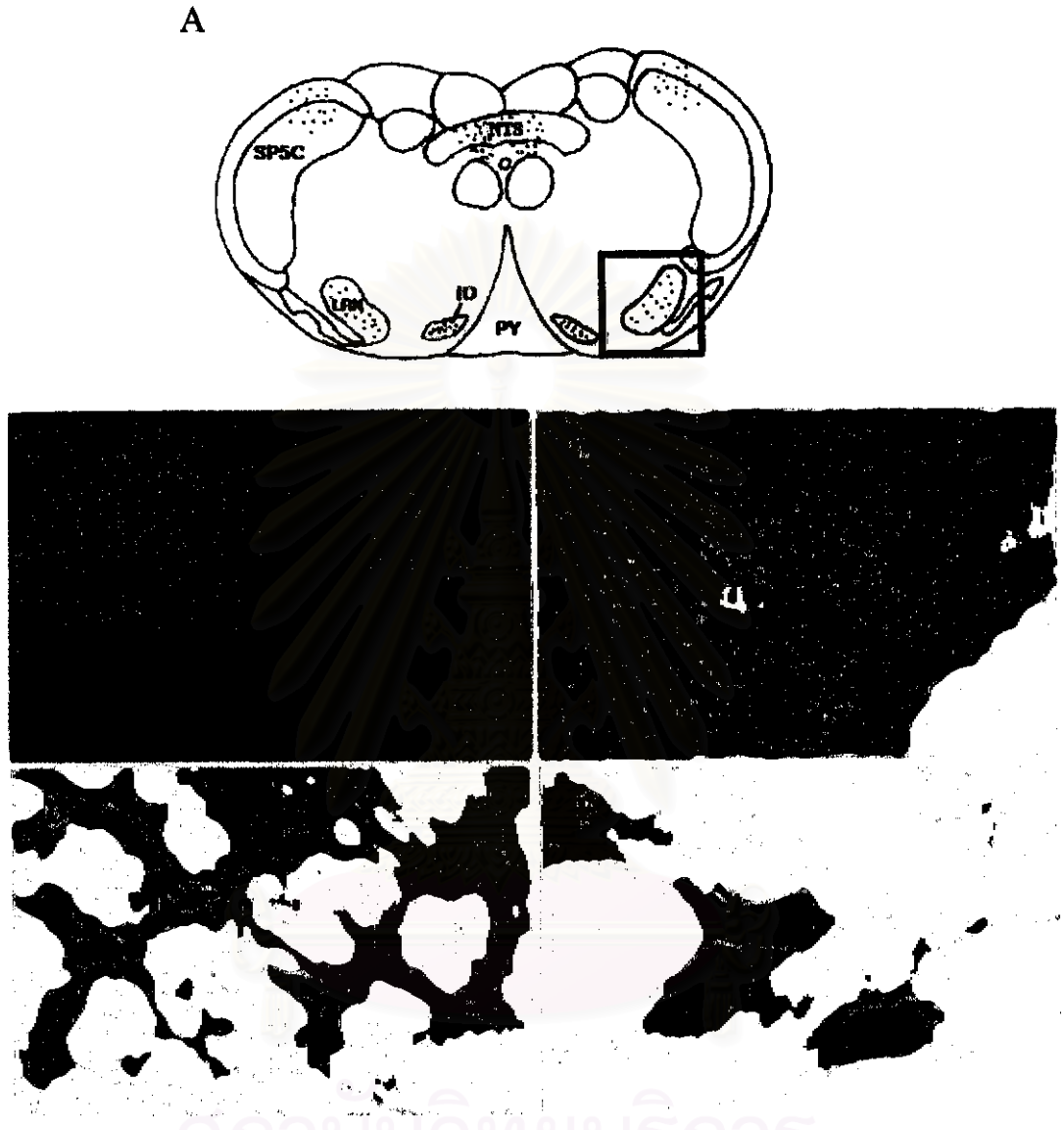


Figure 5.14. Photomicrographs showing the Fos immunoreactivity in LRN of the brainstem of the rat. Fos positive cells (arrow) are easily visible and well differentiated from the background after NTG (10 mg/kg BW) infusion. Box in the schematic drawings indicate the area the respective photographs were taken from. (A) Camera lucida drawing of brainstem of NTG-treated rat, (B) Control (C) NTG-treated rat (x4), (D) higher magnification of (C) (x20) and (E) higher magnification of (C) (x100). (SP5C=spinal trigeminal nucleus caudalis, NTS=nucleus tractus solitarius, LRN=lateral recticular nucleus, IO=inferior olive and PY=pyramidal tract).

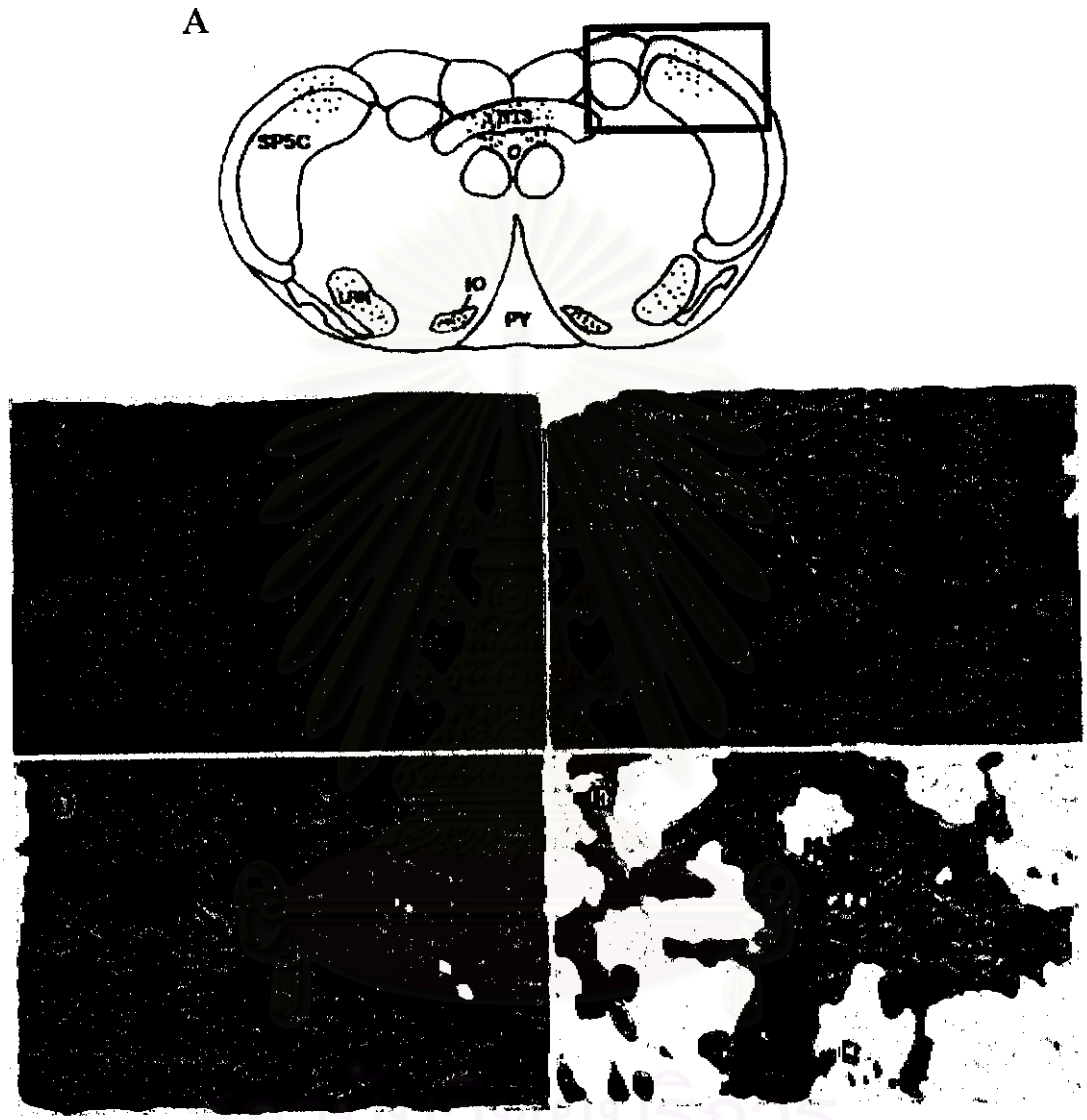


Figure 5.15. Photomicrographs showing the Fos immunoreactivity in TNC of the brainstem of the rat. Fos positive cells (arrow) are easily visible and well differentiated from the background after NTG (10 mg/kg BW) infusion. Box in the schematic drawings indicate the area the respective photographs were taken from. (A) Camera lucida drawing of brainstem of NTG-treated rat, (B) Control (C) NTG-treated rat (x10), (D) higher magnification of (C) (x20) and (E) higher magnification of (C) (x40). (SP5C=spinal trigeminal nucleus caudalis, NTS=nucleus tractus solitarius, LRN=lateral recticular nucleus, IO=inferior olive and PY=pyramidal tract)

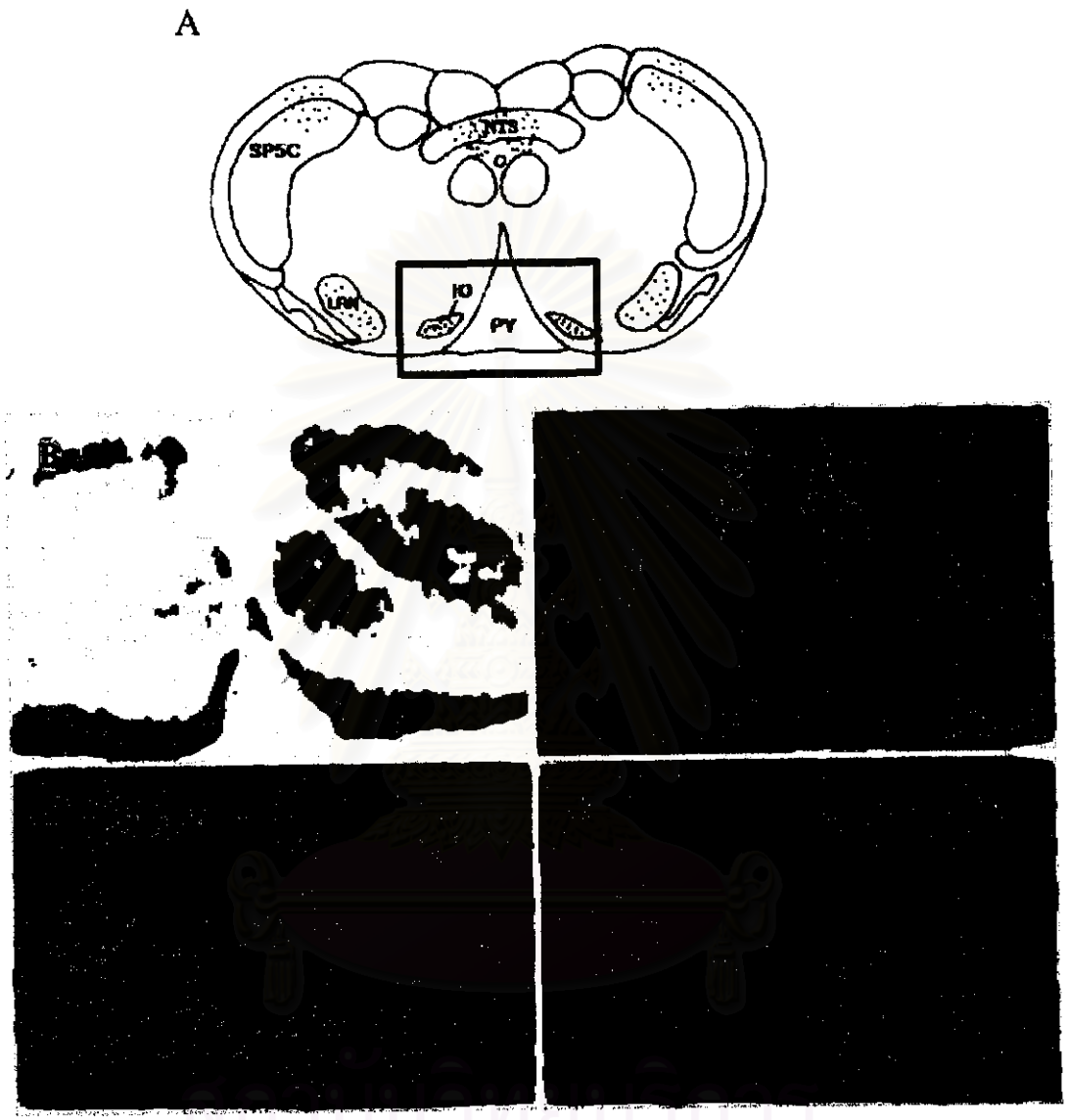


Figure 5.16. Photomicrographs showing the Fos immunoreactivity in IO of the brainstem of the rat. Fos positive cells (arrow) are easily visible and well differentiated from the background after NTG (10 mg/kg BW) infusion. Box in the schematic drawings indicate the area the respective photographs were taken from. (A) Camera lucida drawing of brainstem of NTG-treated rat, (B) Control (C) NTG-treated rat (x4), (D) higher magnification of (C) (x20) and (E) higher magnification of (C) (x40). (SP5C=spinal trigeminal nucleus caudalis, NTS=nucleus tractus solitarius, LRN=lateral recticular nucleus, IO=inferior olive and PY=pyramidal tract)

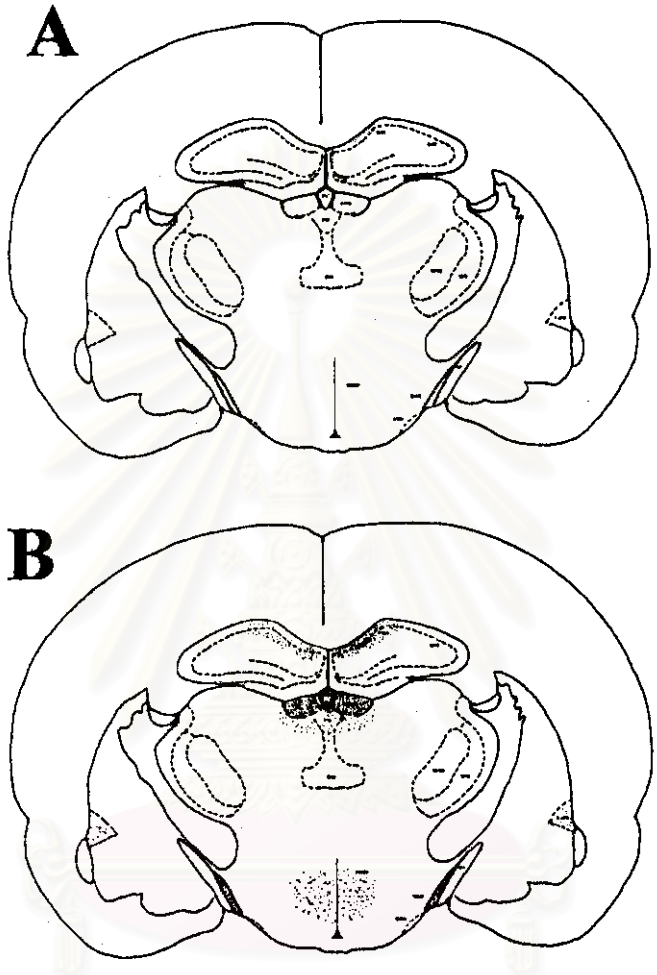


Figure 5.17. Showing the camera lucida drawing of coronal section of the epithalamus in (A) sham rat and (B) NTG-treated rat.

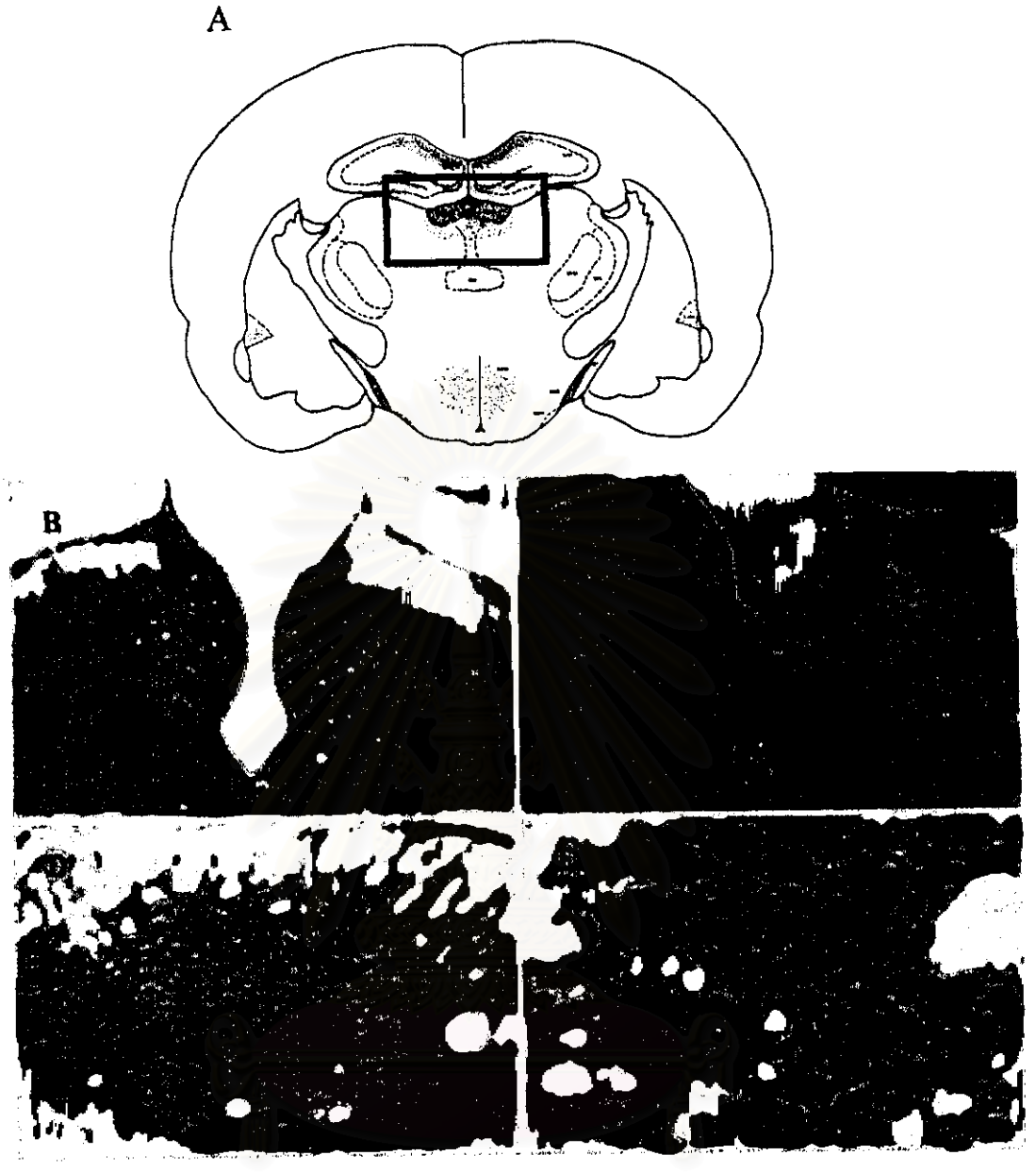


Figure 5.18. Photomicrographs showing the Fos immunoreactivity in habenular of the epithalamus of the rat. Fos positive cells (arrow) are easily visible and well differentiated from the background after NTG (10 mg/kg BW) infusion. Box in the schematic drawings indicate the area the respective photographs were taken from. (A) Camera lucida drawing of epithalamus of NTG-treated rat, (B) Control (C) NTG-treated rat (x4), (D) higher magnification of (C) (x20) and (E) higher magnification of (C) (x40).

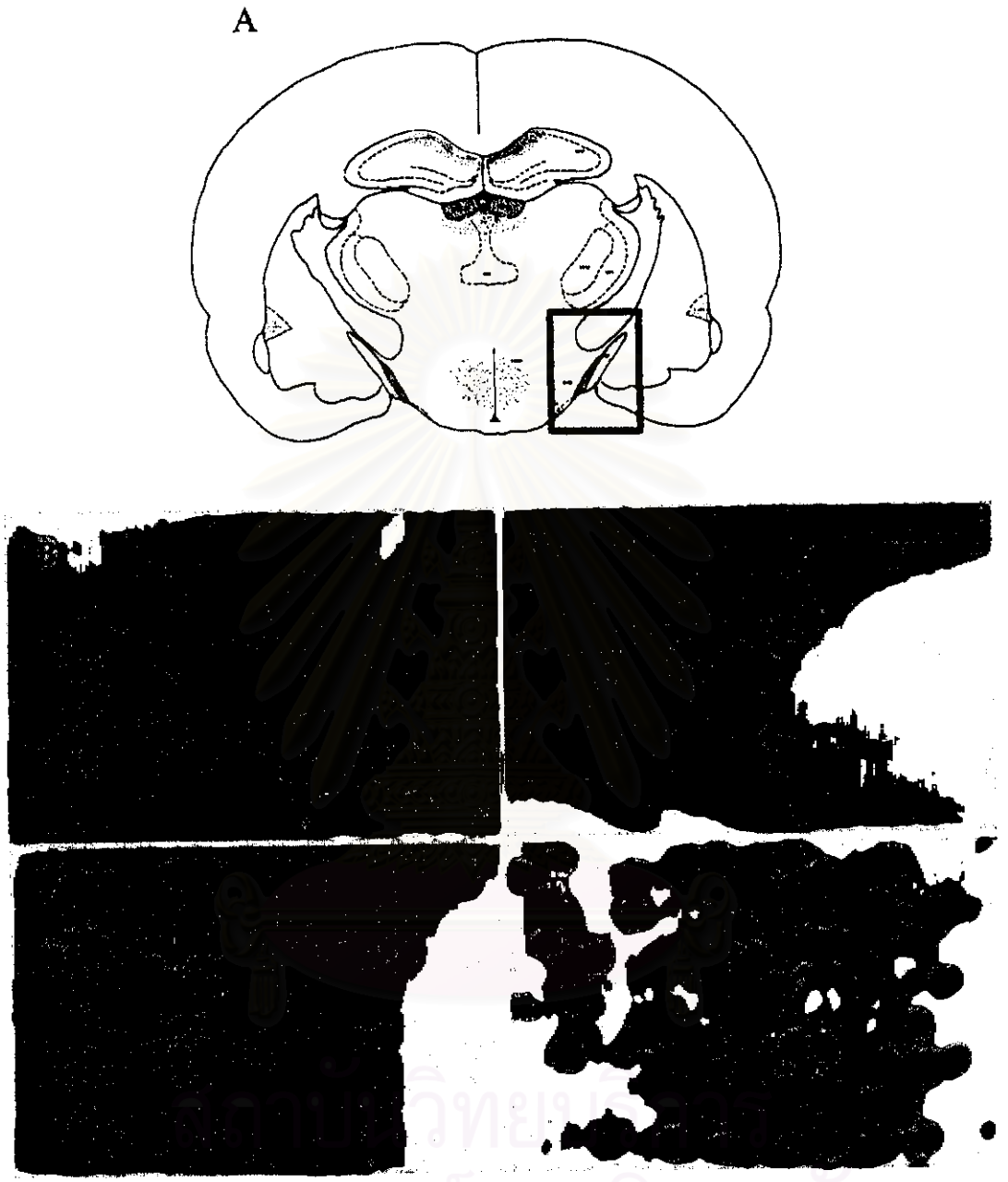


Figure 5.19. Photomicrographs showing the Fos immunoreactivity in supraoptic of the hypothalamus of the rat. Fos positive cells (arrow) are easily visible and well differentiated from the background after NTG (10 mg/kg BW) infusion. Box in the schematic drawings indicate the area the respective photographs were taken from. (A) Camera lucida drawing of hypothalamus of NTG-treated rat, (B) Control (C) NTG-treated rat (x10), (D) higher magnification of (C) (x20) and (E) higher magnification of (C) (x40).

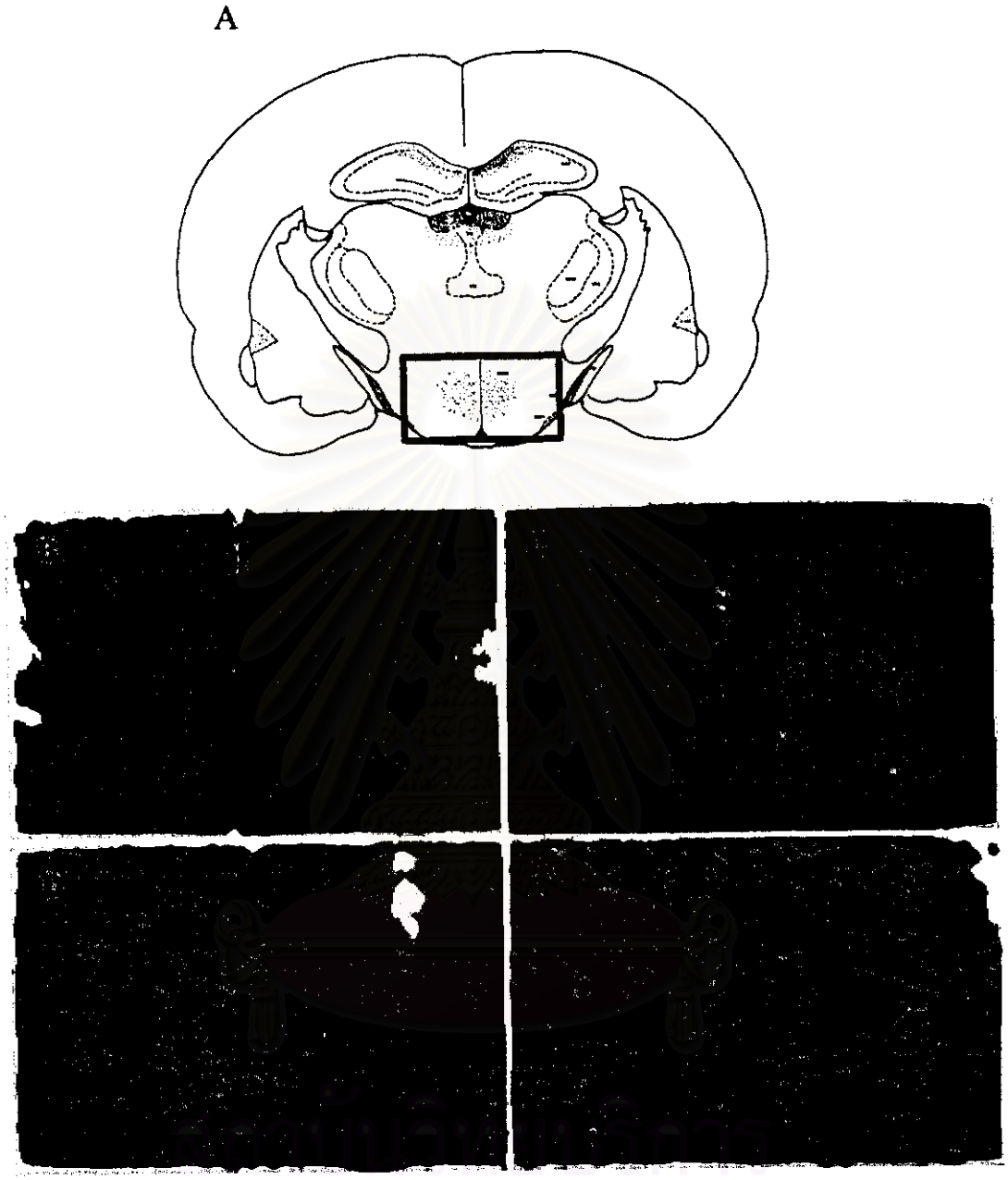


Figure 5.20. Photomicrographs showing the Fos immunoreactivity in paraventricular nuclei of the hypothalamus of the rat. Fos positive cells (arrow) are easily visible and well differentiated from the background after NTG (10 mg/kg BW) infusion. Box in the schematic drawings indicate the area the respective photographs were taken from. (A) Camera lucida drawing of hypothalamus of NTG-treated rat, (B) Control (C) NTG-treated rat (x4), (D) higher magnification of (C) (x10) and (E) higher magnification of (C) (x20).

5. The effect of NTG on pial arterioles (diameter about 10-30 μm) of PCPA-treated rats compared to saline-treated rats.

The videotape recorded from this experiment was played back frame by frame and 6 pial arterioles were randomly selected from each PCPA rat (n=5). Images of 30 selected vessels were digitized and the diameters of these vessels were determined as described in chapter IV. The diameters of these arterioles before and after NTG (8 and 10 mg/kg BW) infusion were demonstrated in Table 5.14 and Table 5.15, respectively. The per cent changes from baseline diameter of both groups were shown in Table 5.16 and Table 5.17, respectively.

The same as that was observed in the arterioles of normal rats. The pial arteriole dilatation was observed almost immediately after NTG infusion, reached its peak at 30 minutes and persisted for at least 60 minutes. The greatest difference was evidenced at 30 minutes post infusion. The magnitude of difference at 30 minutes in rat receiving 8 mg/kg BW of NTG = 42.57 $\pm$ 2.50 per cent (95% CI = 37.45 to 47.69, $P < 0.001$) and rat receiving 10 mg/kg BW of NTG = 62.02 $\pm$ 5.28 per cent (95% CI = 51.22 to 72.82, $P < 0.001$).

Pretreatment with PCPA increased both magnitude and duration of NTG-induced pial arteriole dilatation, especially in the late phase (minute 30 and 60). Per cent changes of pial arteriole diameter at minute 60 in rat receiving 8 mg/kg BW of NTG were 41.10 $\pm$ 2.81 and 18.69 $\pm$ 2.57 for rat with and without PCPA pretreatment, respectively. (magnitude of difference = 22.41 $\pm$ 4.14, 95% CI = 14.13 to 30.70, $P < 0.001$) (Figure 5.21) In rat receiving 10 mg/kg BW of NTG, the per cent changes of pial arteriole diameter at minute 60 were 60.97 $\pm$ 5.07 and 46.60 $\pm$ 6.36 for rat

with and without PCPA pretreatment, respectively. (magnitude of difference = 14.37 $\pm$ 7.85, 95% CI = 1.34 to 30.08, $P < 0.07$). (Figure 5.22) The effect of PCPA upon NTG-induced pial arteriole dilatation was more prominent in rats receiving lower dosage of NTG.

Table 5.14. The effect of nitroglycerin on PCPA-treated rat pial arterioles (diameter about 10-30 μm) before and after 8 mg/kg BW of NTG infusion at 5, 15, 30 and 60 minutes.

Vessels Number	0 min	Diameter after NTG infusion (μm)			
		5 min	15 min	30 min	60 min
1	15	20	20	21	25
2	18	22	24	24	27
3	24	29	32	38	38
4	30	35	40	46	47
5	18	23	25	25	25
6	25	25	30	35	35
7	19	22	23	28	22
8	21	25	25	25	25
9	15	20	20	25	25
10	21	25	27	27	24
11	26	35	41	45	46
12	18	22	28	28	28
13	30	38	45	48	48
14	19	22	25	25	25
15	18	22	26	28	28
16	18	23	25	25	25
17	25	33	35	33	32
18	15	23	23	24	18
19	30	37	40	38	38
20	18	25	26	25	25
21	23	28	35	34	34
22	22	28	29	29	24
23	18	21	24	26	25
24	22	22	30	30	30
25	22	23	27	32	30
26	30	31	39	39	40
27	28	30	39	39	39
28	14	18	18	18	19
29	25	25	30	30	30
30	18	18	22	28	32
Average	21.50	25.67*	29.10*	30.60*	30.30*
S.E.	0.89	1.01	1.30	1.37	1.46

$P < 0.001$ compared to control

Table 5.15. The effect of nitroglycerin on PCPA-treated rat pial arterioles (diameter about 10-30 μm) before and after 10 mg/kg BW of NTG infusion at 5, 15, 30 and 60 minutes.

Vessels Number	0 min	Diameter after NTG infusion (μm)			
		5 min	15 min	30 min	60 min
1	27	33	36	36	36
2	27	33	33	38	40
3	29	35	39	39	39
4	16	24	31	32	30
5	20	29	36	36	40
6	28	42	43	45	45
7	23	30	32	33	35
8	15	20	22	24	24
9	22	29	31	33	33
10	17	25	30	31	30
11	14	19	20	22	24
12	17	19	22	22	23
13	16	28	29	29	29
14	12	23	25	28	25
15	15	30	32	32	33
16	28	32	32	35	35
17	23	39	39	37	31
18	16	28	29	29	29
19	20	27	28	28	28
20	24	30	37	38	38
21	16	18	22	25	27
22	22	30	30	30	31
23	12	22	22	25	22
24	18	26	26	24	22
25	18	32	34	35	36
26	18	23	23	25	25
27	15	28	30	30	31
28	25	32	32	32	31
29	20	24	27	30	30
30	14	17	21	21	19
Average	19.57	27.57*	29.77*	30.80*	30.70*
S.E.	0.92	1.10	1.09	1.06	1.14

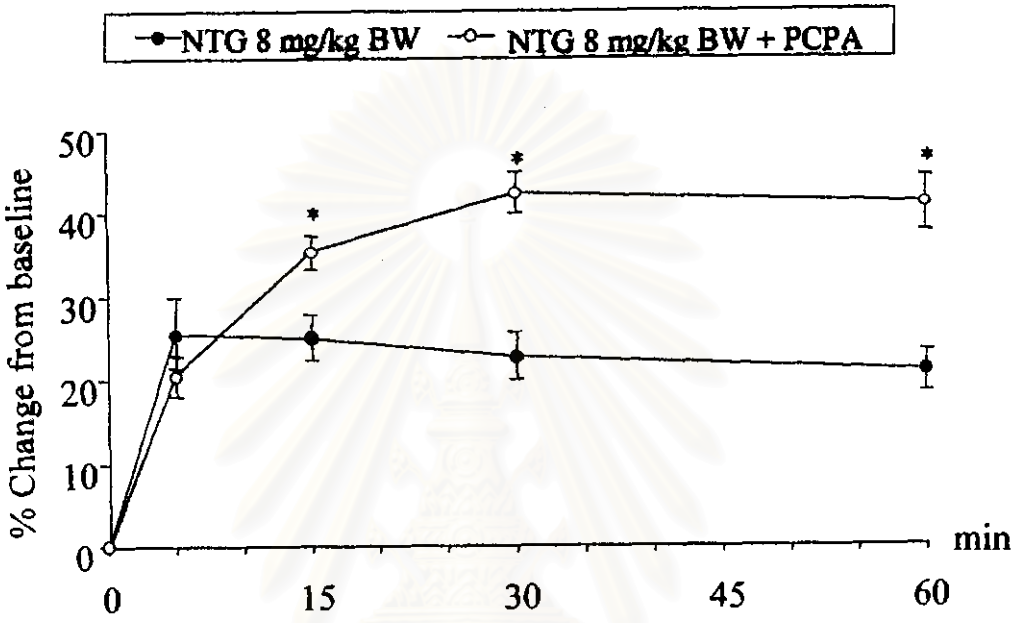
$P < 0.001$ compared to control

Table 5.16. The per cent change from baseline diameter of PCPA-treated rat pial arterioles (diameter about 10-30 μm) after 8 mg/kg BW of NTG infusion at 5, 15, 30 and 60 minutes.

Vessels Number	% Changes after NTG infusion			
	5 min	15 min	30 min	60 min
1	33.33	33.33	40.00	66.67
2	22.22	33.33	33.33	50.00
3	20.83	33.33	58.33	58.33
4	16.67	33.33	53.33	56.67
5	27.78	38.89	38.89	38.89
6	0.00	20.00	40.00	40.00
7	15.79	21.05	47.37	15.79
8	19.05	19.05	19.05	19.05
9	33.33	33.33	66.67	66.67
10	19.05	28.57	28.57	14.29
11	34.62	57.69	73.08	76.92
12	22.22	55.56	55.56	55.56
13	26.67	50.00	60.00	60.00
14	15.79	31.58	31.58	31.58
15	22.22	44.44	55.56	55.56
16	27.78	38.89	38.89	38.89
17	32.00	40.00	32.00	28.00
18	53.33	53.33	60.00	20.00
19	23.33	33.33	26.67	26.67
20	38.89	44.44	38.89	38.89
21	21.74	52.17	47.83	47.83
22	27.27	31.82	31.82	9.09
23	16.67	33.33	44.44	38.89
24	0.00	36.36	36.36	36.36
25	4.55	22.73	45.45	36.36
26	3.33	30.00	30.00	33.33
27	7.14	39.29	39.29	39.29
28	28.57	28.57	28.57	35.71
29	0.00	20.00	20.00	20.00
30	0.00	22.22	55.56	77.78
Average	20.47	35.33	42.57	41.10
S.E.	2.36	1.98	2.50	3.35

Table 5.17. The per cent change from baseline diameter of PCPA-treated rat pial arterioles (diameter about 10-30 μm) after 10 mg/kg BW of NTG infusion at 5, 15, 30 and 60 minutes.

Vessels	% Changes after NTG infusion			
Number	5 min	15 min	30 min	60 min
1	22.22	33.33	33.33	33.33
2	22.22	22.22	40.74	48.15
3	20.69	34.48	34.48	34.48
4	50.00	93.75	100.00	87.50
5	45.00	80.00	80.00	100.00
6	50.00	53.57	60.71	60.71
7	30.43	39.13	43.48	52.17
8	33.33	46.67	60.00	60.00
9	31.82	40.91	50.00	50.00
10	47.06	76.47	82.35	76.47
11	35.71	42.86	57.14	71.43
12	11.76	29.41	29.41	35.29
13	75.00	81.25	81.25	81.25
14	91.67	108.33	133.33	108.33
15	100.00	113.33	113.33	120.00
16	14.29	14.29	25.00	25.00
17	69.57	69.57	60.87	34.78
18	75.00	81.25	81.25	81.25
19	35.00	40.00	40.00	40.00
20	25.00	54.17	58.33	58.33
21	12.50	37.50	56.25	68.75
22	36.36	36.36	36.36	40.91
23	83.33	83.33	108.33	83.33
24	44.44	44.44	33.33	22.22
25	77.78	88.89	94.44	100.00
26	27.78	27.78	38.89	38.89
27	86.67	100.00	100.00	106.67
28	28.00	28.00	28.00	24.00
29	20.00	35.00	50.00	50.00
30	21.43	50.00	50.00	35.71
Average	44.14	56.21	62.02	60.97
S.E.	4.75	5.05	5.28	5.11



* $P < 0.05$ compared to NTG 8 mg/kg BW infusion

Figure 5.21. The per cent change from baseline of rat pial microvessel diameter (% from baseline) in cerebral arteriole (diameter about 10-30 μm) of control and PCPA-treated rats after NTG 8 mg/kg BW) infusion at 5, 15, 30 and 60 minutes.

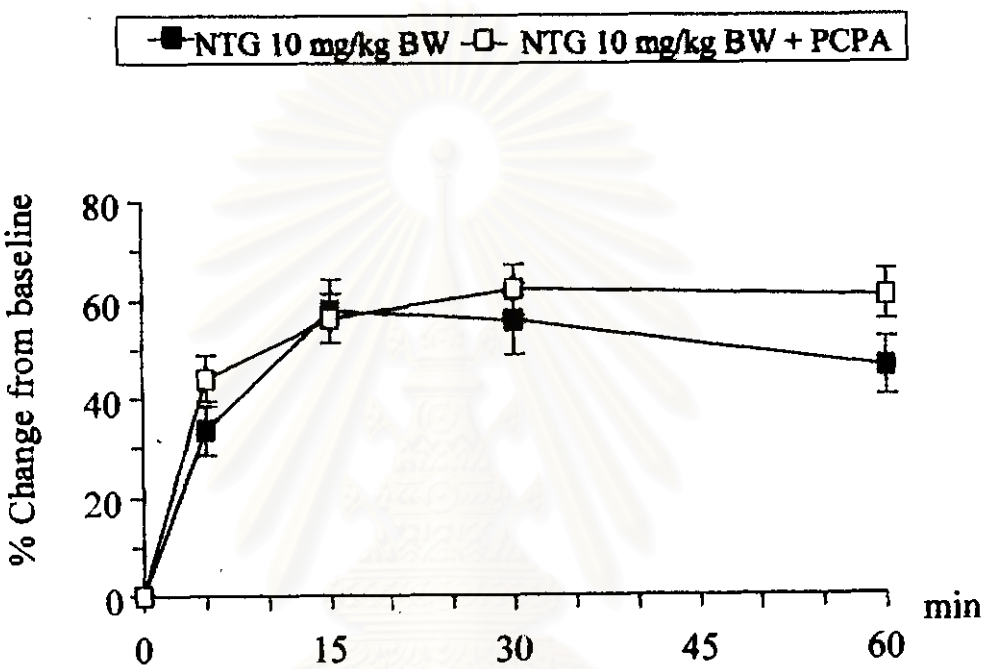


Figure 5.22. The per cent change from baseline of rat pial microvessel diameter (% from baseline) in cerebral arteriole (diameter about 10-30 μm) of control and PCPA-treated rats after NTG 10 mg/kg BW) infusion at 5, 15, 30 and 60 minutes.

6. The effect of NTG on pial arterioles (diameter about 30-60 μm of PCPA-treated rats compared to saline-treated rats.

The videotape recorded from this experiment was played back frame by frame and 3 pial arterioles were randomly selected from each PCPA rat (n=5). Images of 15 selected vessels were digitized and the diameters of these vessels were determined as described in chapter IV. The diameters of these arterioles before and after NTG (8 and 10 mg/kg BW) infusion were demonstrated in Table 5.18 and Table 5.19, respectively. The per cent changes from baseline diameter of both groups were shown in Table 5.20 and Table 5.21, respectively.

The same as that was observed in the arteriole of normal rats. The pial arteriole dilatation was observed almost immediately after NTG infusion, reached its peak at 30 minutes and persisted for at least 60 minutes. The greatest difference was evidenced at 30 minutes post infusion. The magnitude of difference at 30 minutes in rat receiving 8 mg/kg BW of NTG = 23.81 $\pm$ 1.44 per cent (95% CI = 20.72 to 26.91, $P < 0.001$). The magnitude of difference at 60 minutes in rats receiving 10 mg/kg BW of NTG = 31.00 $\pm$ 2.51, 95% CI = 25.68 to 36.44, $P < 0.001$).

Pretreatment with PCPA increased both magnitude and duration of NTG-induced pial arteriole dilatation, especially in the late phase (minute 30 and 60). Per cent changes of pial arteriole diameter at minute 30 in rat receiving 8 mg/kg BW of NTG were 23.81 $\pm$ 1.44 and 13.11 $\pm$ 1.92 for rat with and without PCPA pretreatment, respectively (magnitude of difference = 10.70 $\pm$ 2.40, 95% CI = 5.78 to 15.62, $P < 0.001$). (Figure 5.23) In rat receiving 10 mg/kg BW of NTG, the per cent changes of pial arteriole diameter at minute 60 were 31.06 $\pm$ 2.51 and 34.42 $\pm$ 6.26 for rat

with and without PCPA pretreatment, respectively. (magnitude of difference = 8.62 $\pm$ 6.87, 95% CI = 5.45 to 22.69, P = 0.22) (Figure 5.24) The effect of PCPA upon NTG-induced pial arteriole dilatation was more prominent in rats receiving lower dosage of NTG.

Table 5.18. The effect of nitroglycerin on PCPA-treated rat pial arterioles (diameter about 30-60 μm) before and after 8 mg/kg BW of NTG infusion at 5, 15, 30 and 60 minutes.

Vessels Number	0 min	Diameter after NTG infusion (μm)			
		5 min	15 min	30 min	60 min
1	35	40	40	45	36
2	38	43	47	49	46
3	31	32	39	39	40
4	39	42	44	48	47
5	38	40	40	48	48
6	41	46	46	55	57
7	52	54	57	60	60
8	34	42	42	42	42
9	60	67	67	70	70
10	40	42	46	48	47
11	40	44	46	46	50
12	42	46	46	50	52
13	47	51	53	60	56
14	50	50	60	64	64
15	40	40	44	50	54
Average	41.80	45.27*	47.80*	51.60*	51.27*
S.E.	1.95	2.07	2.08	2.21	2.37

* $P < 0.001$ compared to control

Table 5.19. The effect of nitroglycerin on PCPA-treated rat pial arterioles (diameter about 30-60 μm) before and after 10 mg/kg BW of NTG infusion at 5, 15, 30 and 60 minutes.

Vessels Number	0 min	Diameter after NTG infusion (μm)			
		5 min	15 min	30 min	60 min
1	31	38	45	45	45
2	48	60	60	60	55
3	33	41	42	46	46
4	52	56	58	65	65
5	58	62	64	69	68
6	60	70	70	72	77
7	54	57	60	64	67
8	36	39	39	45	50
9	48	50	53	62	70
10	57	61	70	80	80
11	50	52	60	64	64
12	37	45	50	50	50
13	37	42	45	50	50
14	40	45	48	48	50
15	42	45	47	49	52
Average	45.53	50.87*	54.07	57.93*	59.27*
S.E.	2.48	2.51	2.55	2.87	2.96

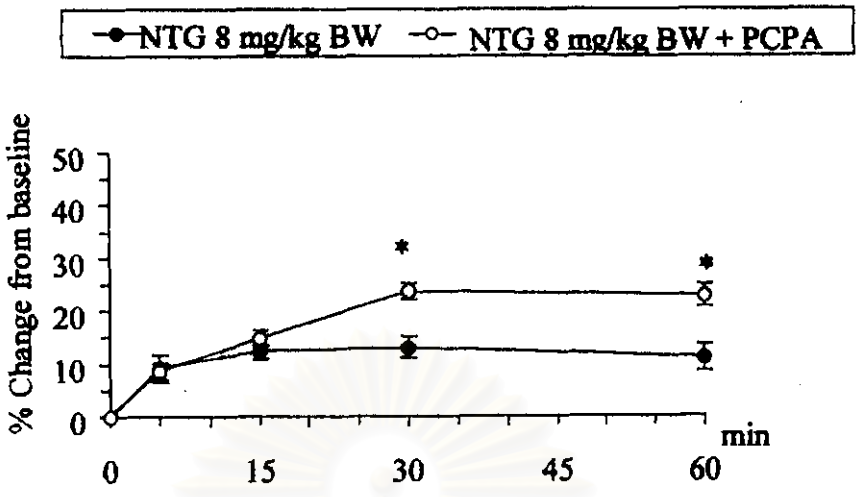
* $P < 0.001$ compared to control

Table 5.20. The per cent change from baseline diameter of PCPA-treated rat pial arterioles (diameter about 30-60 μm) after 8 mg/kg BW of NTG infusion at 5, 15, 30 and 60 minutes.

Vessels Number	% Changes after NTG infusion			
	5 min	15 min	30 min	60 min
1	14.29	14.29	28.57	2.86
2	13.16	23.68	28.95	21.05
3	3.23	25.81	25.81	29.03
4	7.69	12.82	23.08	20.51
5	5.26	5.26	26.32	26.32
6	12.20	12.20	34.15	39.02
7	3.85	9.62	15.38	15.38
8	23.53	23.53	23.53	23.53
9	11.67	11.67	16.67	16.67
10	5.00	15.00	20.00	17.50
11	10.00	15.00	15.00	25.00
12	9.52	9.52	19.05	23.81
13	8.51	12.77	27.66	19.15
14	0.00	20.00	28.00	28.00
15	0.00	10.00	25.00	35.00
Average	8.53	14.74	23.81	22.86
S.E.	1.58	1.54	1.44	2.22

Table 5.21. The per cent change from baseline diameter of PCPA-treated rat pial arterioles (diameter about 30-60 μm) after 10 mg/kg BW of NTG infusion at 5, 15, 30 and 60 minutes.

Vessels Number	% Changes after NTG infusion			
	5 min	15 min	30 min	60 min
1	22.58	45.16	45.16	45.16
2	25.00	25.00	25.00	14.58
3	24.24	27.27	39.39	39.39
4	7.69	11.54	25.00	25.00
5	6.90	10.34	18.97	17.24
6	16.67	16.67	20.00	28.33
7	5.56	11.11	18.52	24.07
8	8.33	8.33	25.00	38.89
9	4.17	10.42	29.17	45.83
10	7.02	22.81	40.35	40.35
11	4.00	20.00	28.00	28.00
12	21.62	35.14	35.14	35.14
13	13.51	21.62	35.14	35.14
14	12.50	20.00	20.00	25.00
15	7.14	11.90	16.67	23.81
Average	12.46	19.82	28.10	31.06
S.E.	1.97	2.67	2.32	2.51



* $P < 0.001$ compared to NTG 8 mg/kg BW infusion

Figure 5.23. The per cent change from baseline of rat pial microvessel diameter (% from baseline) in cerebral arteriole (diameter about 30-60 μ m) of control and PCPA-treated rats after NTG 8 mg/kg BW) infusion at 5, 15, 30 and 60 minutes.

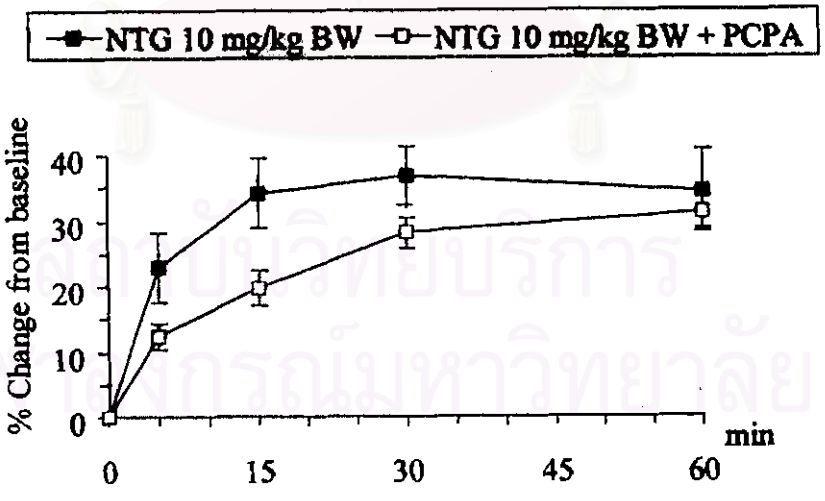


Figure 5.24. The per cent change from baseline of rat pial microvessel diameter (% from baseline) in cerebral arteriole (diameter about 30-60 μ m) of control and PCPA-treated rats after NTG 10 mg/kg BW) infusion at 5, 15, 30 and 60 minutes.

7. The effect of NTG on endothelial ultrastructural changes of cerebral capillaries and arterioles of PCPA-treated rats compared to saline-treated rats.

Microvillous Formation. Table 5.22 showed that the average number of microvilli in cerebral capillaries in rats without PCPA pretreatment were 0.50 ± 0.35, 1.37 ± 0.50 and 2.83 ± 1.66 microvilli per vessel for the control, 8 and 10 mg/kg BW NTG-treated groups, respectively. An greater density of microvilli observed in the 10 mg/kg BW NTG-treated group was statistically significant ($P < 0.05$) as compared with the controls. The average number of microvilli in cerebral capillaries in rat with PCPA pretreatment were 0.85 ± 0.19, 3.10 ± 1.37 and 2.08 ± 0.38 microvilli per vessel for the control, 8 and 10 mg/kg BW NTG-treated group, respectively. Pretreatment with PCPA resulted in a tendency to increase density of capillary microvilli especially in the rats receiving NTG though without any statistical significance. The average number of microvilli in cerebral arterioles in rats without PCPA pretreatment were 1.28 ± 0.86, 3.40 ± 1.74 and 9.16 ± 3.54 microvilli per vessel for the control, 8 and 10 mg/kg BW NTG-treated group, respectively. A greater density of microvilli observed in NTG (10 mg/kg BW)-treated group was statistically significant ($P < 0.05$) as compared with the controls. The average number of microvilli in cerebral arterioles in rat with PCPA pretreatment were 2.26 ± 0.60, 9.52 ± 3.94 and 10.86 ± 4.48 microvilli per vessel for control, 8 and 10 mg/kg BW NTG-treated groups, respectively. (Figure 5.25) In contrast to capillary, pretreatment with PCPA resulted in a greater density of arteriole microvilli especially in the group receiving 8 mg/kg BW of NTG. (Figure 5.26) The average difference between the number of microvilli observed in rats receiving 8

mg/kg BW NTG infusion with and without PCPA pretreatment was 6.12 ± 1.87 microvilli/vessel (95% CI= 3.52 to 12.20, $P < 0.0048$).

Number of Pinocytic Vesicles. The most obvious change observed in NTG-infused groups was an increase in density of pinocytic vesicles. In the control group, only low number of pinocytic vesicles were observed in endothelial cells of the cerebral microvessels. Pretreatment with PCPA did not cause any significant change in the pinocytic vesicular density. On the contrary, these vesicles became more abundant in endothelial cell of cerebral microvessels taken from rats receiving 10 mg/kg BW of NTG (Table 5.23). Increased pinocytosis was demonstrated on both the luminal and abluminal surface of cerebral microvessels. The average density of pinocytic vesicles in cerebral capillaries were 9.92 ± 2.75, 8.56 ± 3.74 and 27.28 ± 2.76 vesicle/μm^2, for control, 8 and 10 mg/kg BW of NTG, respectively. No significant change was evident when density of pinocytic vesicle from the group receiving 8 mg/kg BW of NTG and the controls were compared. The average density of pinocytic vesicles in cerebral capillaries of PCPA-treated rats were 7.52 ± 1.57, 23.12 ± 4.17 and 18.72 ± 6.11 vesicle/μm^2, for control, 8 and 10 mg/kg BW of NTG, respectively (Figure 5.27). In capillaries pretreatment with PCPA increased the sensitivity in this ultrastructural change as shown by marked increase in number of vesicle in rats receiving PCPA pretreatment and 8 mg/kg BW NTG infusion. The average difference between the value observed in rat receiving 8 mg/kg BW NTG infusion with and without PCPA pretreatment was 14.56 ± 2.40 vesicle/μm^2 (95% CI=6.74 to 22.38, $P < 0.001$). The average density of pinocytic vesicles in cerebral arterioles were 9.68 ± 0.70, 9.86 ± 1.14 and 28.84 ± 1.56 vesicle/μm^2, for the control, 8 and 10 mg/kg BW of NTG, respectively. No significant change was evident when density of

pinocytic vesicle from the group receiving 8 mg/kg BW of NTG and the controls were compared. The average density of pinocytic vesicle in cerebral arterioles of PCPA-treated rats were 7.90 ± 1.25, 26.48 ± 3.93 and 25.12 ± 5.78 vesicle/μm^2, for the control, 8 and 10 mg/kg BW of NTG, respectively (Figure 5.28). In arterioles pretreatment with PCPA increased the sensitivity of this ultrastructural change as shown by a marked increase in number of vesicle (26.48 ± 1.76 vesicle/μm^2) in rats receiving PCPA pretreatment and 8 mg/kg BW NTG infusion. The average difference between the value observed in rats receiving 8 mg/kg BW NTG infusion with and without PCPA pretreatment was 16.62 ± 2.18 vesicle/μm^2 (95%CI = 9.51 to 23.73, $P < 0.001$).

Mitochondrial Diameter. The average mitochondrial diameter in cerebral microvessels taken from the control group was 154.76±39.95 nm for capillaries and 167.42 ± 38.67 nm for arterioles. Therefore, the mitochondria with diameter greater than 235 nm in capillaries and 245 nm in arterioles (mean+2SD) were defined as swollen. The percentage of swollen mitochondria was significantly greater in NTG (10 mg/kg BW)-treated groups as compared to the controls (31.18 % and 4.5 % for capillaries of NTG-treated and control groups, respectively, odd ratio 9.63, 95 % CI = 3.01 to 34.13, $P < 0.001$ and 23.53 % and 3.16 % for arterioles of NTG-treated and control groups, respectively (odd ratio 9.78, 95 % CI=4.11 to 23.91, $P < 0.001$) (Table 5.12). No significant change was evident when mitochondrial diameter from the control pretreatment with PCPA and the normal controls were compared. (Table 5.24) A greater percentage of swollen mitochondria of endothelial capillaries were evidenced in the 8 mg/kg BW NTG-treated rats with PCPA pretreatment as compared to those receiving the same dose of NTG but without PCPA pretreatment (32.41 and 17.17 % respectively, $P < 0.02$) (Table 5.25). A

greater percentage of swollen mitochondria of endothelial arterioles were evidenced in the 8 mg/kg BW NTG-treated rats with PCPA pretreatment as compared to those receiving the same dose of NTG but without PCPA pretreatment (35.16 and 15.68 % respectively, $P < 0.02$) (Table 5.26). No significant difference was observed when value taken from 10 mg/kg BW NTG-treated group with and without PCPA pretreatment were compared 37.11 and 31.18 %, respectively, $P = 0.39$ for capillaries and 28.42 and 23.53%, respectively, $P = 0.45$ for arterioles. (Table 5.26)

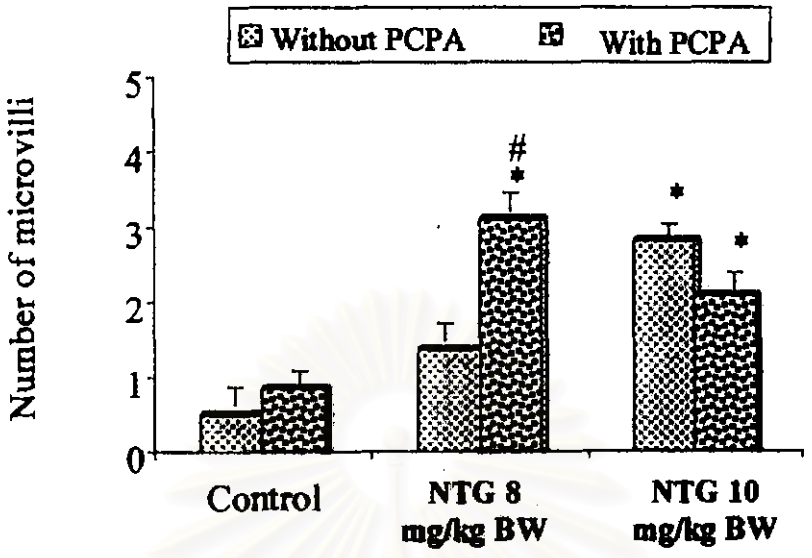
Other Morphological Changes. Other abnormal features observed in the NTG-treated groups included edema of periarteriolar astrocytic footplate and dome-like protrusion of endothelial cells into the luminal surface. Both features were not evidenced in the control group. The number of arterioles with periarteriolar astrocytic foot plate swelling observed in 50 vessels in the control and 10 mg/kg BW NTG-treated groups was 2 and 29 vessels respectively (odd ratio = 22.15, 95% CI = 4.50 to 47.64, $P < 0.001$). No significant change was observed when value taken from animals with and without PCPA pretreatment was compared.

Table 5.22. The mean±SD of the number of microvilli/vessel in the endothelial cell of cerebral capillaries (diameter range from 5-10 μm) (n=100) and arterioles (diameter range from 15-30 μm) (n=50) obtained from control, NTG (8 or 10 mg/kg BW)-treated rats, NTG (8 or 10 mg/kg BW)-treated PCPA rats.

Group	Microvilli (number/vessel)	
	Capillary	Arteriole
Control	0.50 ± 0.35	1.28 ± 0.86
PCPA	0.85 ± 0.19	2.26 ± 0.60
NTG 8 mg/kg BW	1.37 ± 0.50	3.40 ± 1.74
NTG 8 mg/kg BW + PCPA	3.10 ± 1.37 #*	9.52 ± 3.94 #*
NTG 10 mg/kg BW	2.83 ± 1.66*	9.16 ± 3.54*
NTG 10 mg/kg BW + PCPA	2.08 ± 0.38*	10.86 ± 4.48*

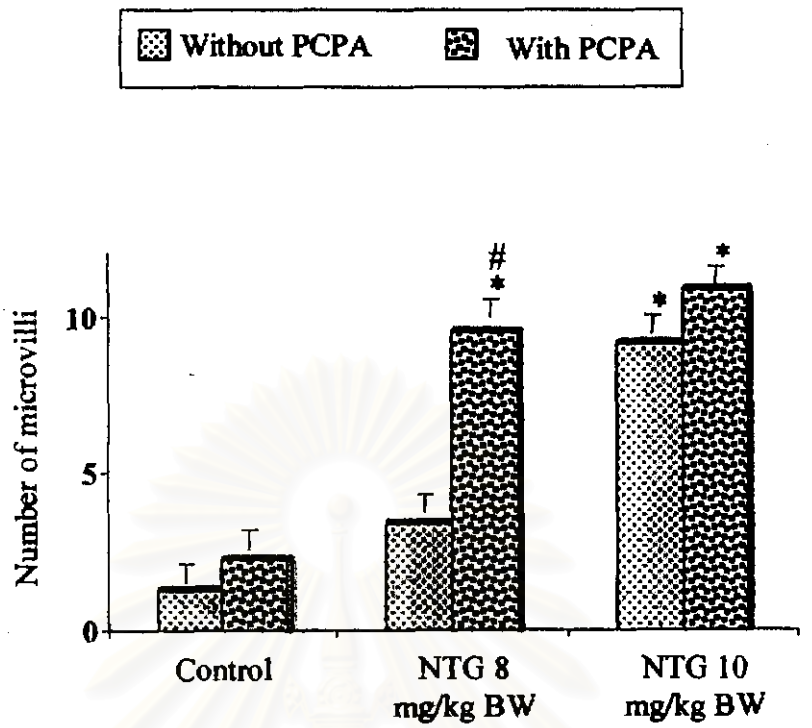
* $P < 0.05$ compared to control

# $P < 0.05$ compared to NTG 8 mg/kg BW



* $P < 0.05$ compared to control
\# $P < 0.05$ compared to NTG 8 mg/kg BW without PCPA

Figure 5.25 Bar graph showing the average number of microvilli in cerebral capillaries with/without PCPA pretreatment after NTG (8 or 10 mg/kg BW) infusion.



* $P < 0.05$ compared to control
\# $P < 0.05$ compared to NTG 8 mg/kg BW without PCPA

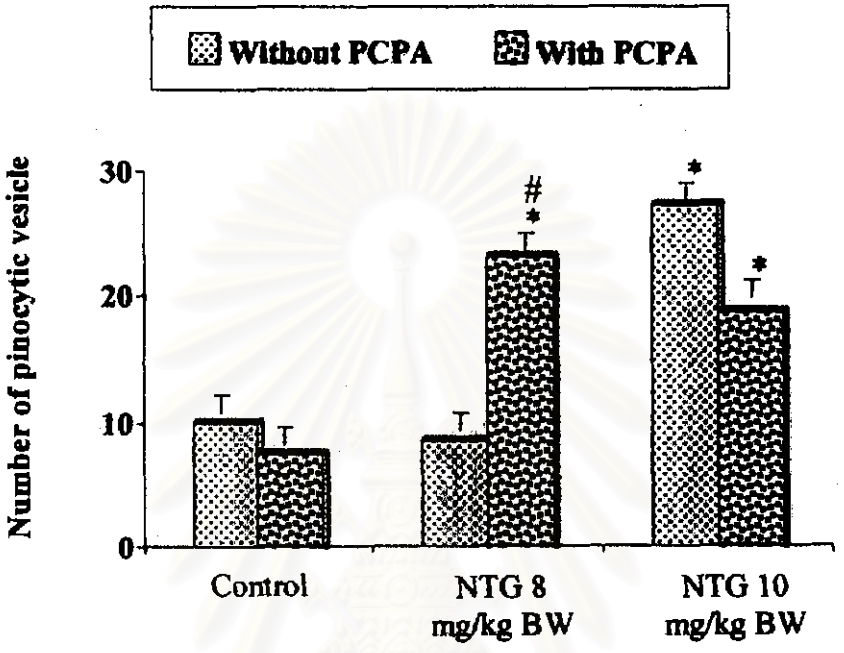
Figure 5.26 Bar graph showing the average number of microvilli in cerebral arterioles with/without PCPA pretreatment after NTG (8 or 10 mg/kg BW) infusion.

Table 5.23. The mean±SD of the number of pinocytic vesicle/μm^2 in the endothelial cell of cerebral capillaries (diameter range from 5-10 μm) and arterioles (diameter range from 15-30 μm) (n=25) that obtained from control, NTG (8 or 10 mg/kg BW)-treated rats, and NTG (8 or 10 mg/kg BW)-treated PCPA rats.

Group	pinocytic vesicle(number/μm^2)	
	Capillary	Arteriole
Control	9.92 ± 2.75	9.68 ± 1.56
PCPA	7.52 ± 1.57	7.90 ± 1.25
NTG 8 mg/kg BW	8.56 ± 3.74	9.86 ± 2.55
NTG 8 mg/kg BW + PCPA	23.12 ± 4.17 #*	26.48 ± 3.93 #*
NTG 10 mg/kg BW	27.28 ± 2.76*	28.84 ± 3.49*
NTG 10 mg/kg BW + PCPA	18.72 ± 6.11*	25.12 ± 5.78*

* $P < 0.05$ compared to control

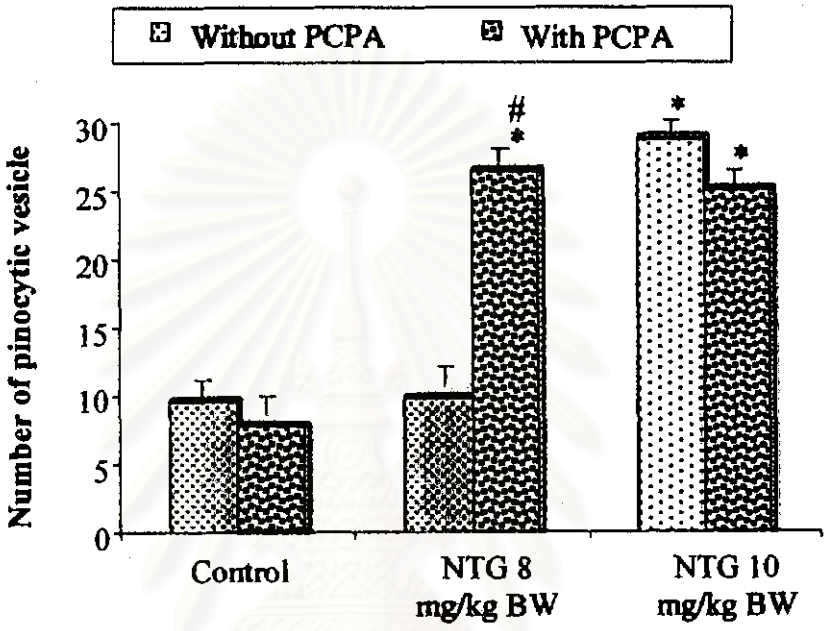
# $P < 0.05$ compared to NTG 8 mg/kg BW



* $P < 0.05$ compared to control

\# $P < 0.05$ compared to NTG 8 mg/kg BW without PCPA

Figure 5.27 Bar graph showing the average number of pinocytic vesicle in cerebral capillaries with/without PCPA pretreatment after NTG (8 or 10 mg/kg BW) infusion.



* $P < 0.05$ compared to control

\# $P < 0.05$ compared to NTG 8 mg/kg BW without PCPA

Figure 5.28 Bar graph showing the average number of pinocytic vesicle in cerebral arterioles with/without PCPA pretreatment after NTG (8 or 10 mg/kg BW) infusion.

Table 5.24. The number of the mitochondrial changes of control and control PCPA-treated rats.

a) capillary

b) arteriole

Significance of difference was determined by chi-square test.

(a)

Number	Mitochondria		Total
	Normal	Abnormal	
Control	85	4	89
PCPA	88	9	97

Odd ratio = 2.27

95% confidential interval (0.58 to 8.75)

$P < 0.20$

(b)

Number	Mitochondria		Total
	Normal	Abnormal	
Control	92	3	95
PCPA	73	9	87

Odd ratio = 3.78

95% confidential interval (0.89 to 18.34)

$P < 0.39$

Table 5.25. The number of the mitochondrial changes of PCPA-treated/ untreated rats after NTG 8 mg/kg BW infusion.

a) capillary

b) arteriole

Significance of difference was determined by chi-square test.

(a)

Groups	Mitochondria		Total
	Normal	Swollen	
NTG 8 mg/kg BW	82	17	99
NTG 8 mg/kg BW+PCPA	73	35	108

Odd ratio = 2.31

95% confidence interval (1.14 to 4.72)

$p < 0.02$

(b)

Groups	Mitochondria		Total
	Normal	Swollen	
NTG 8 mg/kg BW	86	16	102
NTG 8 mg/kg BW+PCPA	59	32	91

Odd ratio = 2.92

95% confidence interval (1.40 to 6.14)

$P < 0.02$

Table 5.26 The number of the mitochondrial changes of PCPA-treated/ untreated rats after NTG 10 mg/kg BW infusion.

c) capillary

d) arteriole

Significance of difference was determined by chi-square test.

(a)

Groups	Mitochondria		Total
	Normal	Swollen	
NTG 10 mg/kg BW	64	29	93
NTG 10 mg/kg BW+PCPA	61	36	97

Odd ratio = 1.30

95% confidence interval (0.68 to 2.49)

$P < 0.39$

(b)

Groups	Mitochondria		Total
	Normal	Swollen	
NTG 10 mg/kg BW	65	20	85
NTG 10 mg/kg BW+PCPA	68	27	95

Odd ratio = 1.92

95% confidence interval (0.63 to 2.67)

$P < 0.46$

8. The effect of NTG evoked fos immunoreactivity in brainstem, epithalamus and hypothalamus of NTG-treated PCPA rats.

As shown in camera lucida drawings of coronal section through the brainstem (Figure 5.29). In sham control PCPA, only scattered immunoreactive neurons were observed in the brainstem segments. In NTG (10 mg/kg BW) infusion group, extensive Fos immunoreactivity were observed in several brainstem nuclei of rat pretreated with/without PCPA. Numerous positive cells were observed in NTS of PCPA-treated rats bilaterally (Figure 5.30). Moderate number of labelled cells were also found bilaterally in LRN (Figure 5.31) and in TNC of PCPA-pretreated rats (Figure 5.32). Finally, the PCPA-treated rats displayed bilateral Fos immunoreactivity in the inferior olive after NTG infusion (Figure 5.33).

In epithalamus and hypothalamus, as shown in camera lucida drawings of coronal sections through the epithalamus (Figure 5.34). Fos-like immunoreactivity was found bilaterally and most intensely within cells of the epithalamus such as habenular (Figure 5.35) of PCPA-pretreated rats after 2 hours NTG infusion. In hypothalamus, fos-like immunoreactive cells were also found in supraoptic (Figure 5.36), paraventricular nuclei (Figure 5.37). On the contrary, only scattered immunoreactive neurons were observed in the same area of control PCPA rats (without NTG infusion).

There were no apparent differences in the number of Fos between NTG-treated with and without PCPA pretreatment (Figure 5.38).

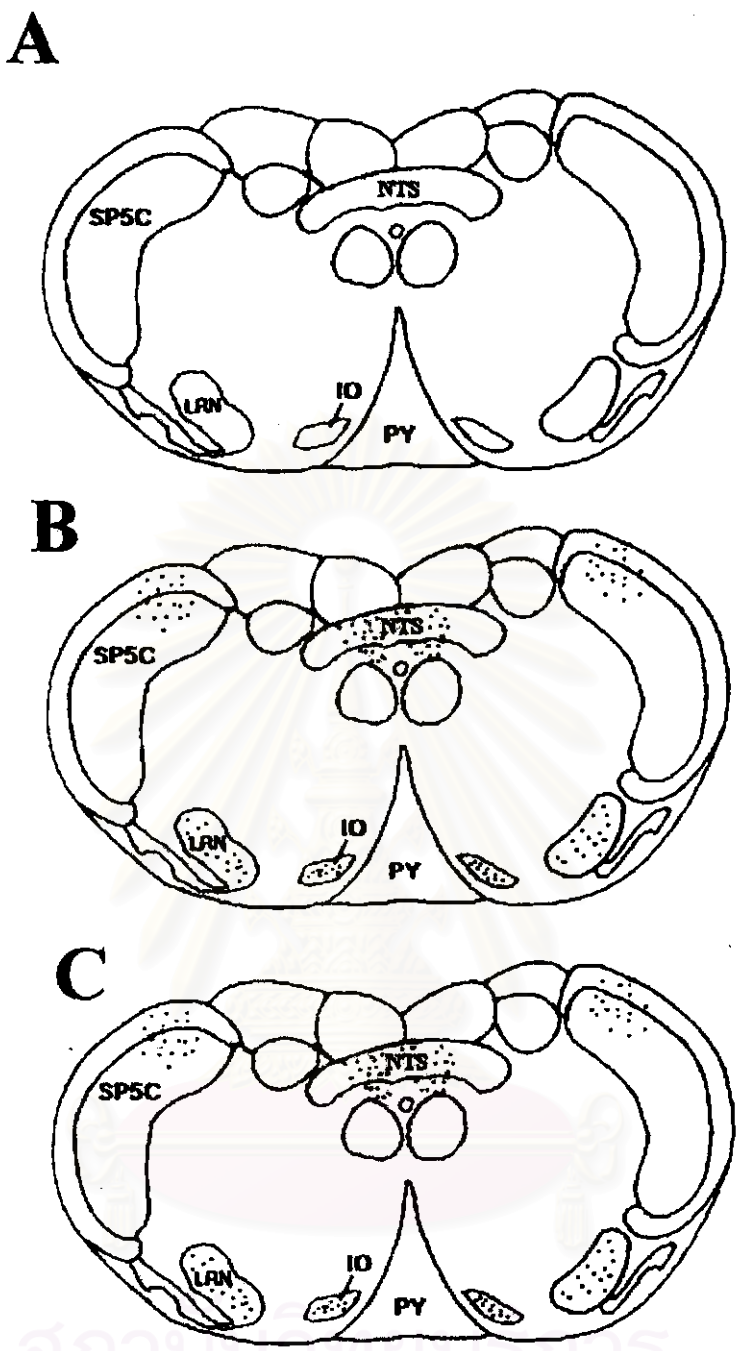


Figure 5.29 Showing the camera lucida drawings of coronal section of the brainstem in (A) sham rat, (B) NTG (10 mg/kg BW)-treated rat and (C) NTG (10 mg/kg BW)-treated PCPA rat. (SP5C=spinal trigeminal nucleus caudalis, NTS=nucleus tractus solitarius, LRN=lateral recticular nucleus, IO=inferior olive and PY=pyramidal tract)

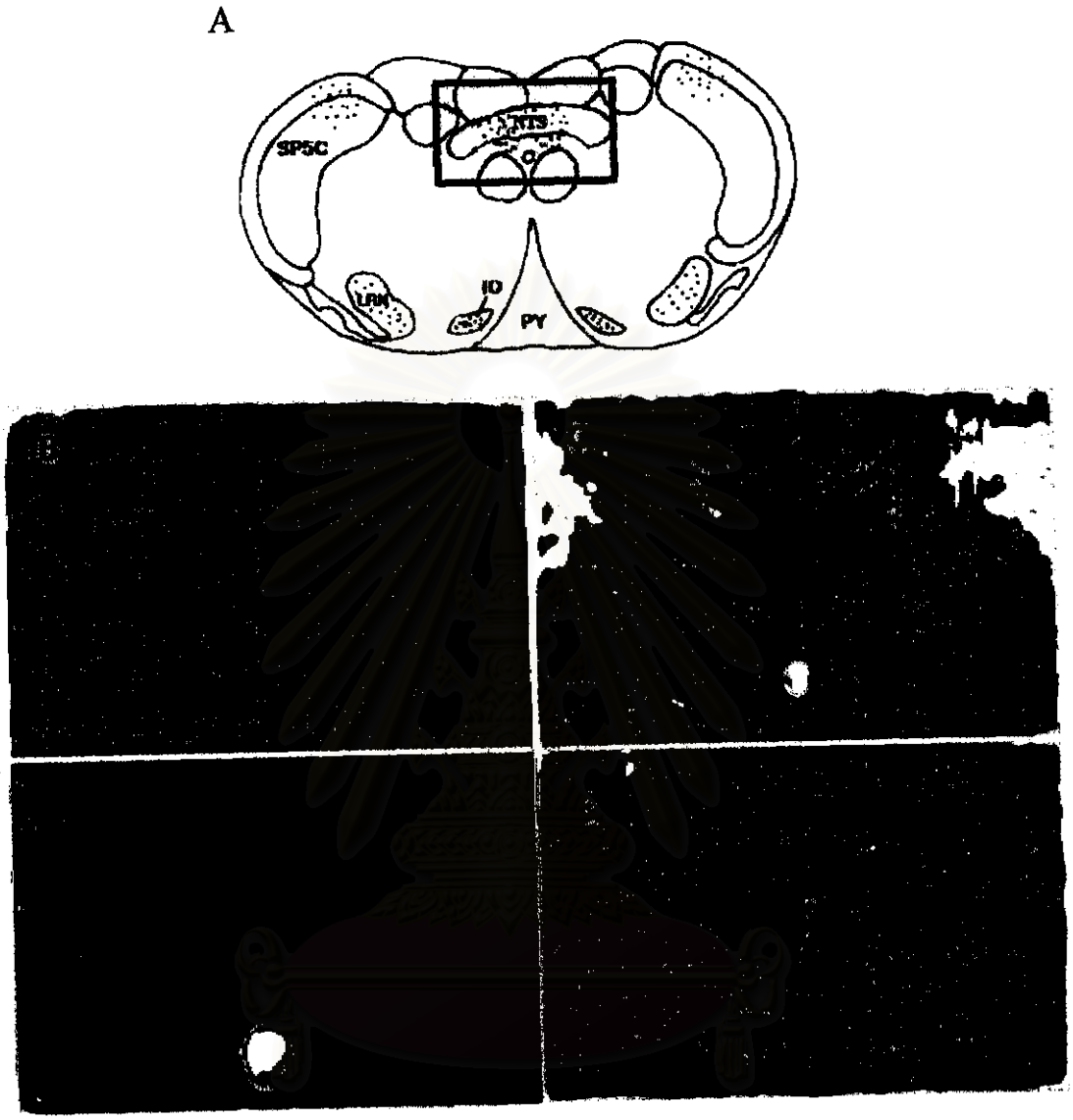


Figure 5.30. Photomicrographs showing the Fos immunoreactivity in NTS of the brainstem of the rat. Fos positive cells (arrow) are easily visible and well differentiated from the background after NTG (10 mg/kg BW) infusion. Box in the schematic drawings indicate the area the respective photographs were taken from. (A) Camera lucida drawing of brainstem of NTG-treated rat, (B) Control (C) NTG-treated rat (x4), (D) NTG-treated PCPA rat (x4) and (E) higher magnification of (D) (x40). (SP5C=spinal trigeminal nucleus caudalis, NTS=nucleus tractus solitarius, LRN=lateral recticular nucleus, IO=inferior olive and PY=pyramidal tract)

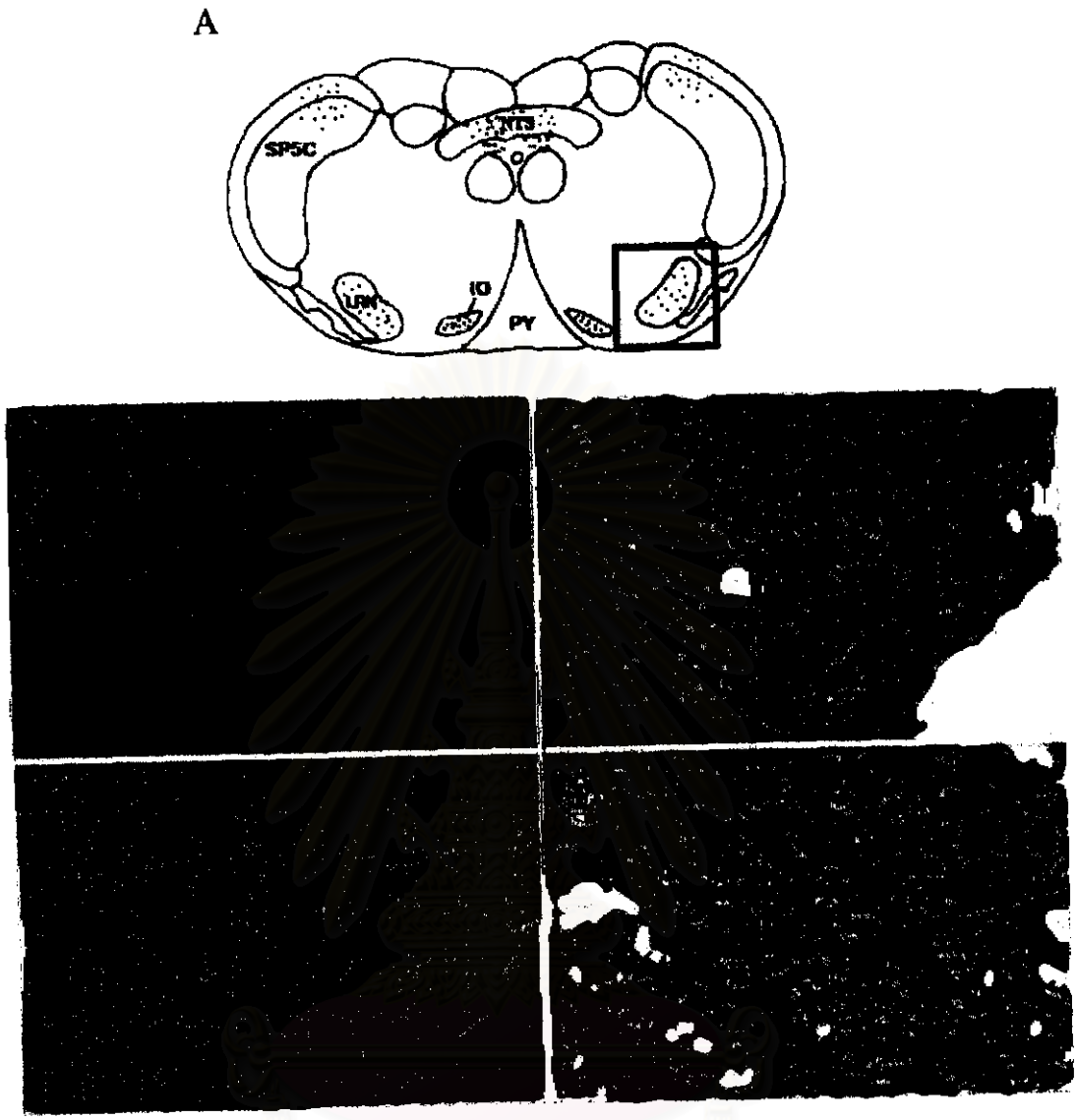


Figure 5.31. Photomicrographs showing the Fos immunoreactivity in LRN of the brainstem of the rat. Fos positive cells (arrow) are easily visible and well differentiated from the background after NTG (10 mg/kg BW) infusion. Box in the schematic drawings indicate the area the respective photographs were taken from. (A) Camera lucida drawing of brainstem of NTG-treated rat, (B) Control (C) NTG-treated rat (x4), (D) NTG-treated PCPA rat (x4) and (E) higher magnification of (D) (x20). (SP5C=spinal trigeminal nucleus caudalis, NTS=nucleus tractus solitarius, LRN=lateral recticular nucleus, IO=inferior olive and PY=pyramidal tract)

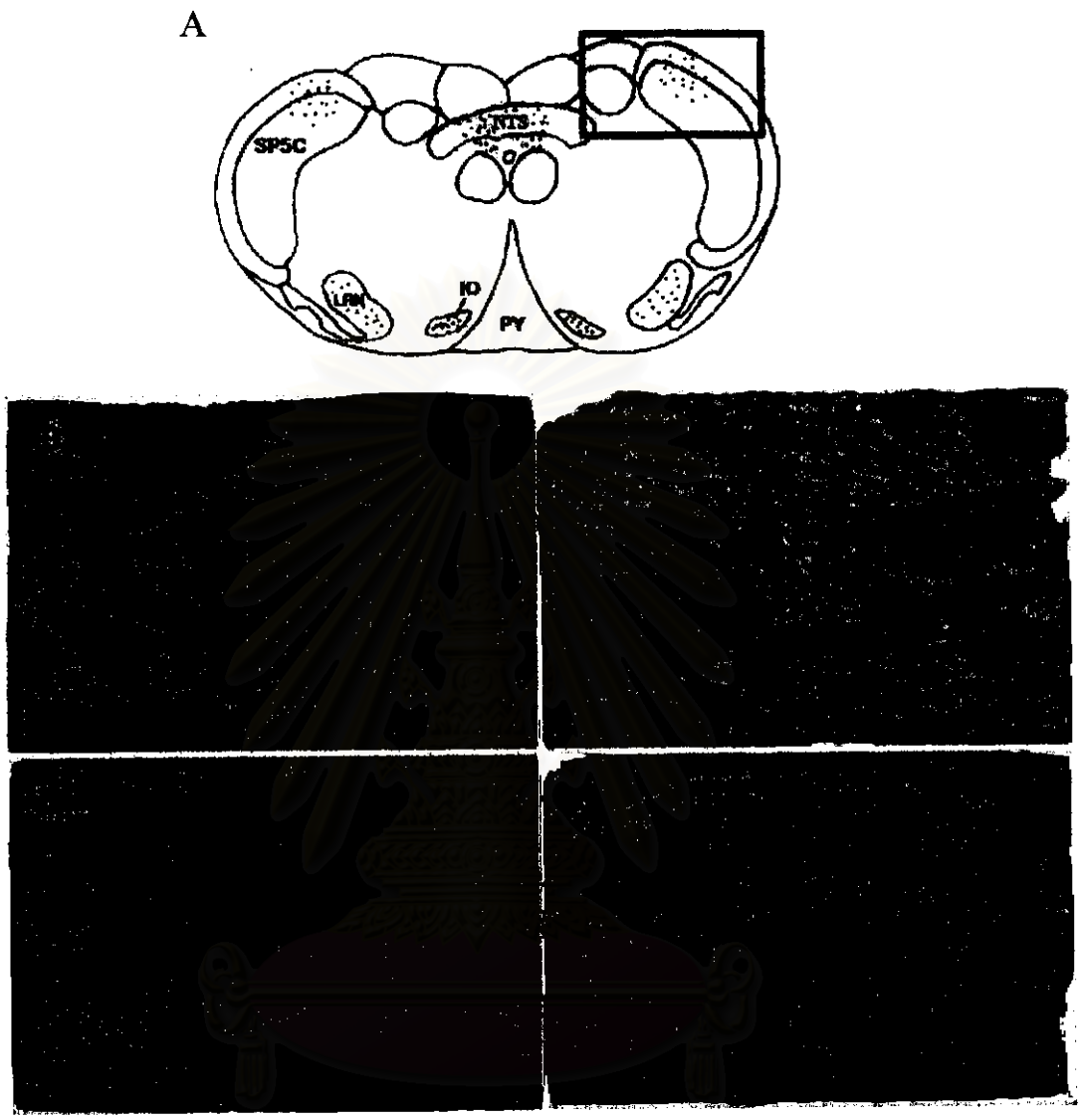


Figure 5.32. Photomicrographs showing the Fos immunoreactivity in TNC of the brainstem of the rat. Fos positive cells (arrow) are easily visible and well differentiated from the background after NTG (10 mg/kg BW) infusion. Box in the schematic drawings indicate the area the respective photographs were taken from. (A) Camera lucida drawing of brainstem of NTG-treated rat, (B) Control (C) NTG-treated rat (x10), (D) NTG-treated PCPA rat (x10) and (E) higher magnification of (D) (x20). (SP5C=spinal trigeminal nucleus caudalis, NTS=nucleus tractus solitarius, LRN=lateral recticular nucleus, IO=inferior olive and PY=pyramidal tract)

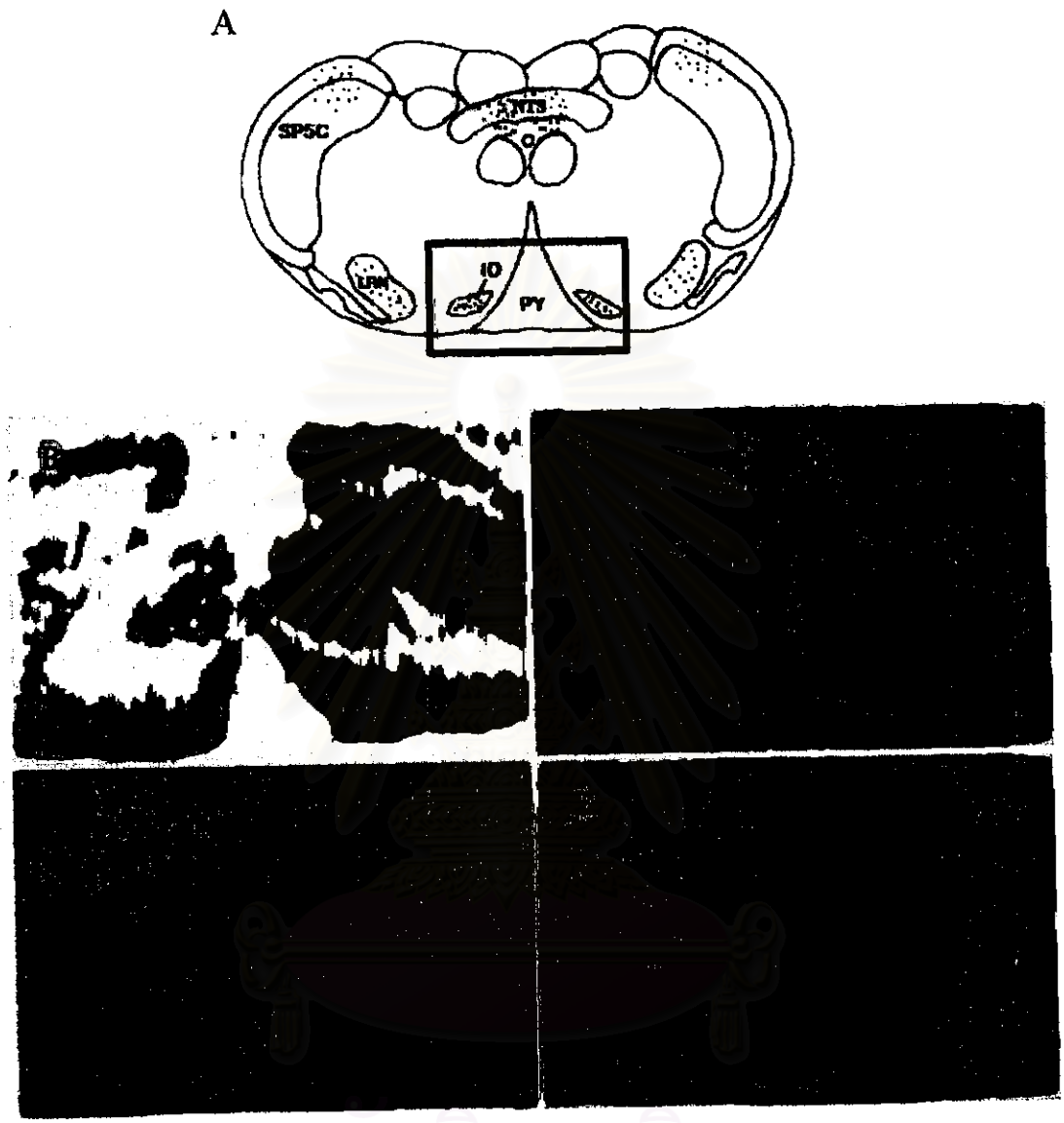


Figure 5.33. Photomicrographs showing the Fos immunoreactivity in IO of the brainstem of the rat. Fos positive cells (arrow) are easily visible and well differentiated from the background after NTG (10 mg/kg BW) infusion. Box in the schematic drawings indicate the area the respective photographs were taken from. (A) Camera lucida drawing of brainstem of NTG-treated rat, (B) Control (C) NTG-treated rat (x4), (D) NTG-treated PCPA rat (x10) and (E) higher magnification of (D) (x20). (SP5C=spinal trigeminal nucleus caudalis, NTS=nucleus tractus solitarius, LRN=lateral recticular nucleus, IO=inferior olive and PY=pyramidal tract)

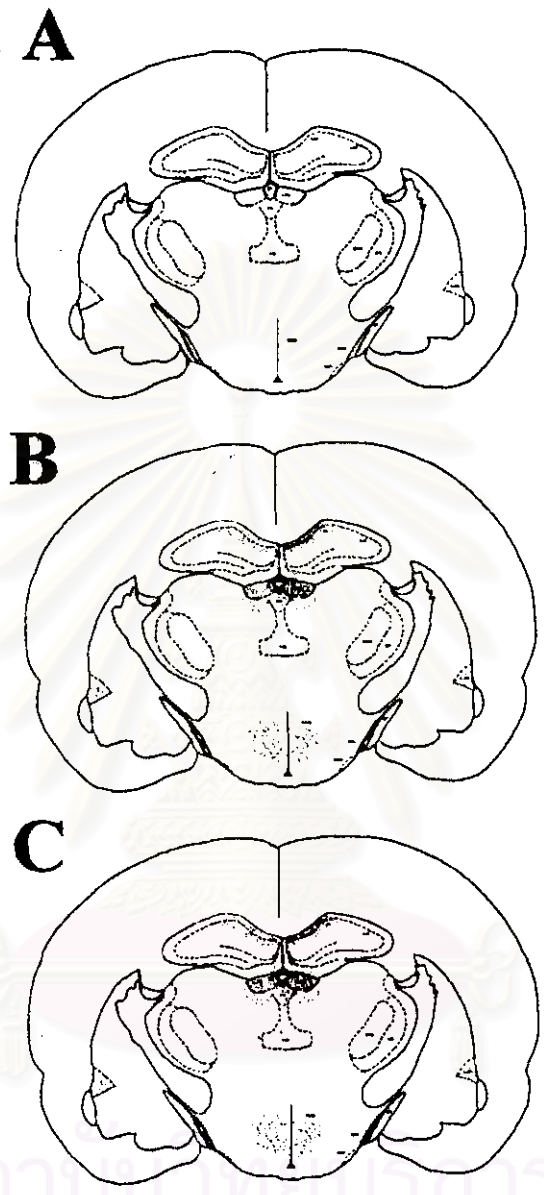


Figure 5.34. The camera lucida drawings of coronal section of the epithalamus in (A) sham rat, (B) NTG (10 mg/kg BW)-treated rat and (C) NTG (10 mg/kg BW)-treated PCPA rat.

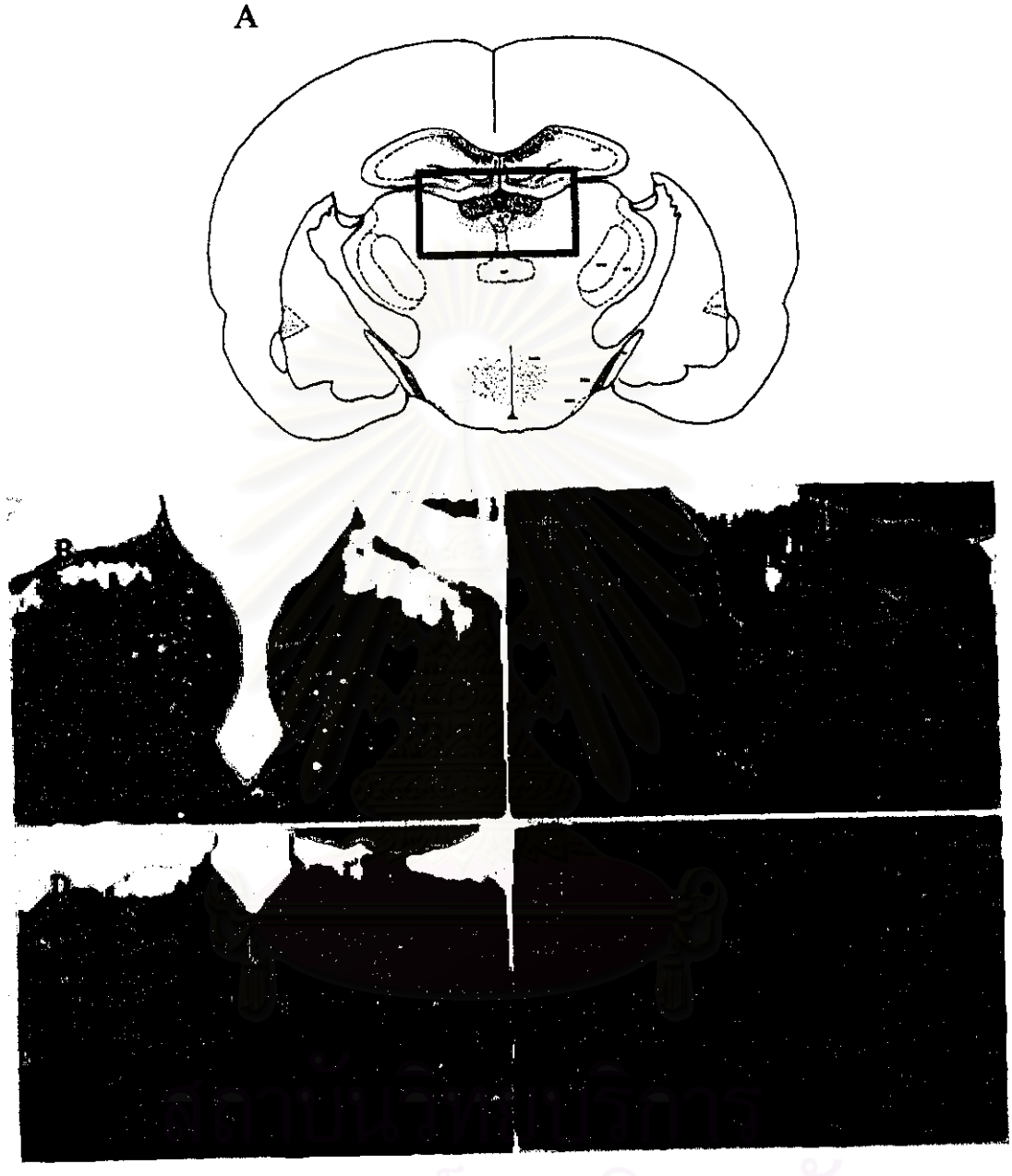


Figure 5.35. Photomicrographs showing the Fos immunoreactivity in habenular of the epithalamus of the rat. Fos positive cells (arrow) are easily visible and well differentiated from the background after NTG (10 mg/kg BW) infusion. Box in the schematic drawings indicate the area the respective photographs were taken from. (A) Camera lucida drawing of epithalamus of NTG-treated rat, (B) Control (C) NTG-treated rat (x4), (D) NTG-treated PCPA rat (x4) and (E) higher magnification of (D) (x20).

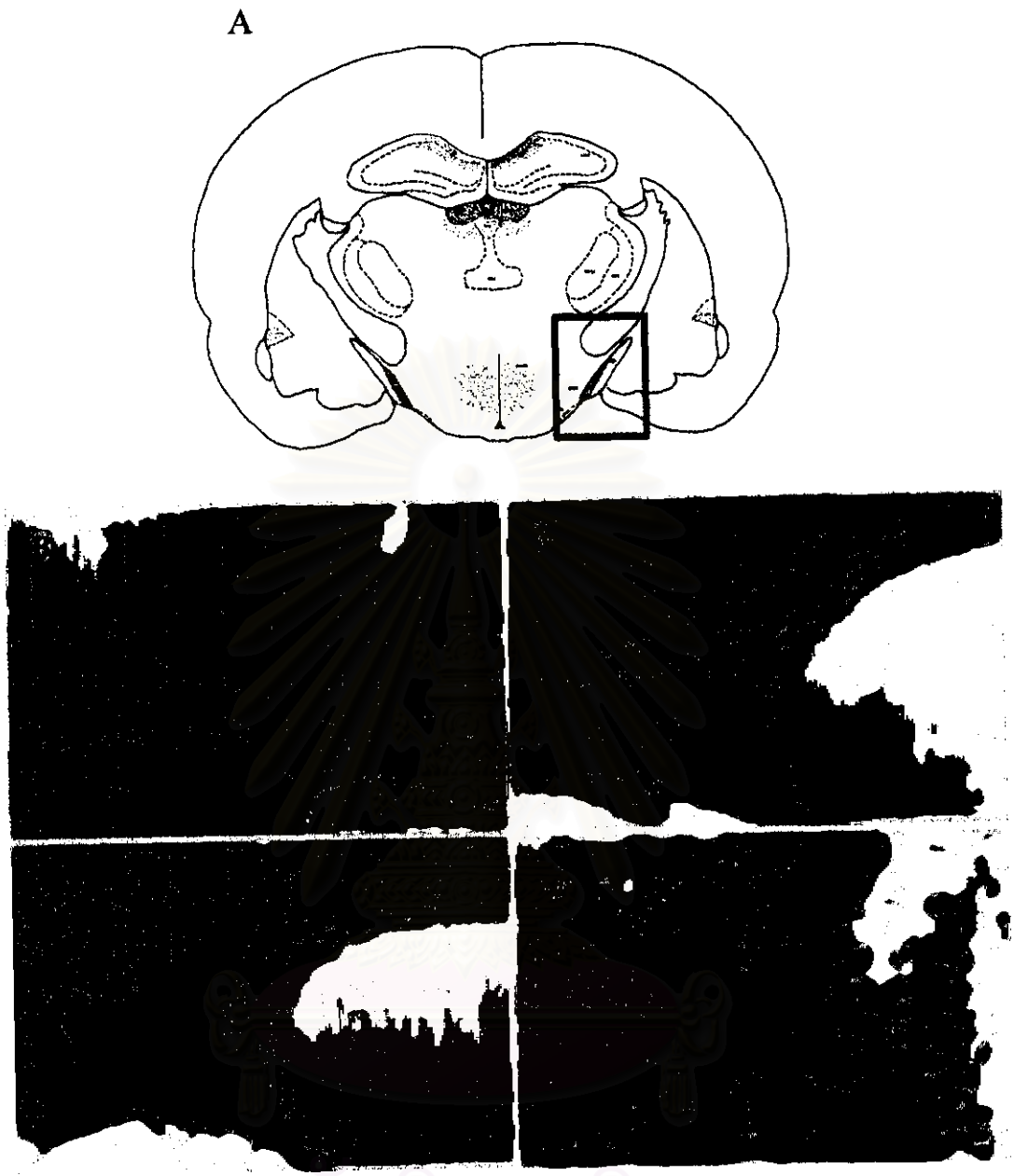


Figure 5.36. Photomicrographs showing the Fos immunoreactivity in supraoptic of the hypothalamus of the rat. Fos positive cells (arrow) are easily visible and well differentiated from the background after NTG (10 mg/kg BW) infusion. Box in the schematic drawings indicate the area the respective photographs were taken from. (A) Camera lucida drawing of hypothalamus of NTG-treated rat, (B) Control (C) NTG-treated rat (x4), (D) NTG-treated PCPA rat (x4) and (E) higher magnification of (D) (x20).

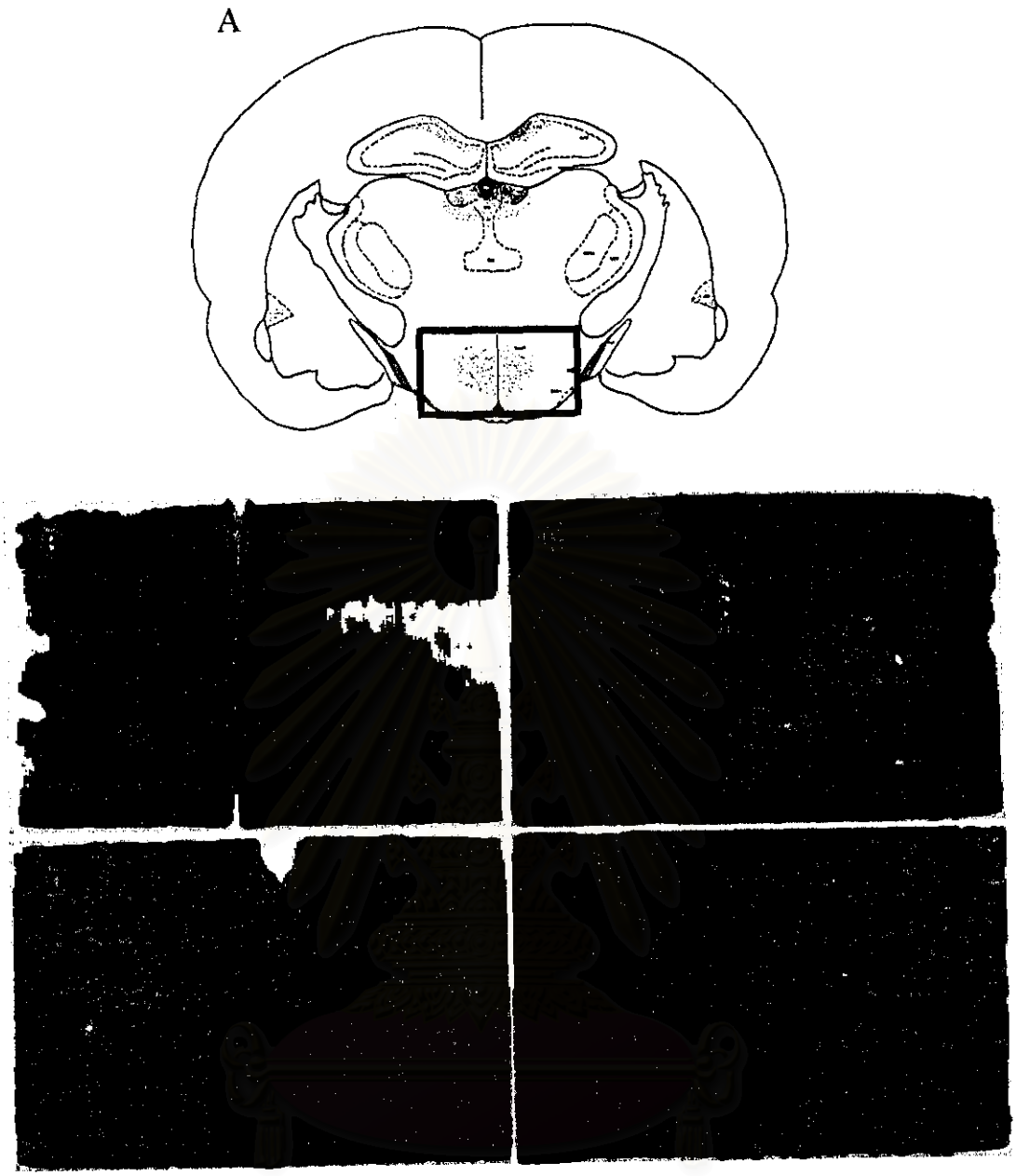


Figure 5.37. Photomicrographs showing the Fos immunoreactivity in paraventricular nuclei of the hypothalamus of the rat. Fos positive cells (arrow) are easily visible and well differentiated from the background after NTG (10 mg/kg BW) infusion. Box in the schematic drawings indicate the area the respective photographs were taken from. (A) Camera lucida drawing of hypothalamus of NTG-treated rat, (B) Control, (C) NTG-treated rat (x4), (D) NTG-treated PCPA rat (x4) and (E) higher magnification of (D) (x20).

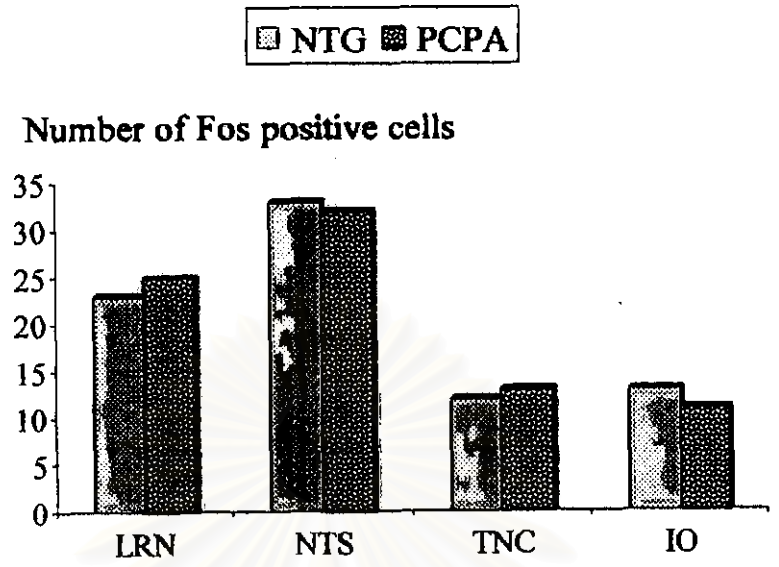


Figure 5.38 Illustrated the mean ± SD of fos-positive cell in lateral reticular nuclei (LRN), nucleus tractus solitarius (NTS), trigeminal nucleus caudalis and inferior olive (IO) of PCPA with/without pretreatment rats after NTG 10 mg/kg infusion.