CHAPTER IV

EFFECT OF STEVIOSIDE ON RENAL FUNCTION

Introduction

The effect of SVS on renal functions has been recently determined. The natriuretic and diuretic effect of SVS without a change of GFR but increase in renal plasma flow (RPF) were noted (Melis and Sainati ,1991a, b) . Controversial results were also demonstrated. SVS induced a greater reduction of urine excretion in accompany with the elevation of plasma BUN and creatinine in rats subjected to 4.1 g/kg.BW of SVS orally(Panichkul et al.,1988). Proximal tubular damages were apparent in rats treated with SVS subcutaneously (Toskulkao et al,1994). Therefore, the experiment was undertaken to evaluate the effect of various doses of SVS intravenous infusion and intubation on renal function in rats.

Materials and methods

The studies were performed on male Wistar rat weighing 250-300 g. The experiments were divided into two series.

Series I Effect of SVS on renal function.

The animals were divided into 5 groups according to dose and route of SVS administration.

Group I (control) After animal preparation as described in chapter III, the animals received normal saline (NSS) intravenously without SVS throughout experiment with the rate 1%kg.BW. Five 30-min period of experiment was established. Blood and urine samples were collected in each period to determine renal hemodynamics and tubular function throughout experiment. Blood pressure and heart rate were also recorded.

Group II (200 mg/kg.BW of SVS infusion) The general protocols were similar to group I. After the first control period, SVS (150 mg/ml in NSS) was intravenously infused as a priming dose of 200 mg/kg.BW. ,and then followed by continuous infusion of 200 mg/kg.BW/hr. Two 30-min periods of SVS infusion and further two 30-min periods of recovery were performed to evaluate renal renal function

Group III (150 mg/kg.BW. of SVS infusion) The similar procedure as group II was performed except that SVS (150 mg/ml) was priming with the dose of 150 mg/kg.BW. and continuously infused with the rate of 150 mg/kg.BW./hr.

Group IV (100 mg/kg.BW. of SVS infusion) The similar procedure as group II was performed except that SVS (150 mg/ml) 100 mg/kg.BW. was priming and followed by 100 mg/kg.BW./hr.

Group V (2 g/kg.BW of SVS intubation) The general protocols were explained in chapter III. In this group, rats were divided into 2 subgroups, control and SVS intubation group.

Renal hemodynamics and tubular function were determined in all experimental groups as described in chapter III.

After finishing in each experiment, renal tissues were collected for histopathological examination

Series II Determination of plasma volume (PV) and blood volume (BV) in response to SVS infusion.

This experiment was underwent to clarify whether hypotensive effect of SVS was the result from the reduction of plasma volume. Plasma volume and blood volume were determined by dye dilution method. The animals were divided into 2 groups, control and SVS infusion group. Five minutes after SVS infusion of 200 mg/kg.BW.(150 mg/ml) , 0.3 ml of Evan's blue dye (1 gm%) was slowly intravenously injected. The subsequent blood samples were withdrawn in every 5 min period up to 20 min. SVS was still infused throughout the experiment with the rate of 200 mg/kg.BW./hr.

Statistics

All values were reported as mean±SEM. Statistical analysis was determined using both paired and unpaired t-test. All values were considered to be significant difference from control period or group at the level of $P<0.05$.

Results

Effect of SVS on general circulation

The alteration of mean arterial pressure (MAP) and heart rate (HR) are shown in figure 4.1 and 4.2. No changes of MAP were appeared in all periods of control group. In contrast, SVS infusion resulted the reduction of MAP to the lowest level in the dose-dependent manner. The minimum level of blood pressure following SVS infusion was exhibited within 5-7 min. MAP was significantly reduced from 119±3 to 86±6 mmHg ($P<0.001$) within 5 min following 200 mg/kg.BW of SVS administration (group II). Similar to group II, group III rats received 150 mg/kg.BW. of SVS infusion also caused the reduction of MAP but to a lesser extent. MAP decreased from 124±3 to 100±4 mmHg ($P<0.001$). The lowest dose of SVS infusion (100 mg/kg.BW.) also produced the fall of MAP from 113±3 to the lowest value of 107±5 mmHg ($P<0.05$). After a transitory decrease of MAP, it gradually increased but remained lower than that of control level. During the subsequent and recovery period, MAP was still decreased whereas it was returned to normal level in the group IV. Slightly change of MAP was apparent in rats treated with 2 g/kg.BW of SVS orally for 6 hours (table 4.7). MAP were declined from 119±2 in control group to 115±1 mmHg ($P<0.05$) in SVS intubation group.

An alteration of heart rate (HR) in response to SVS administration is demonstrated in figure 4.2 and table 4.7. In control group, HR was quite stable except the last period of recovery that it was slightly augmented. The first 30-min of SVS infusion significant elevation of HR in all groups were apparent ($P<0.01$). The elevation of HR was remained even the 60 min of SVS infusion and the recovery period which was corresponding to the lower blood pressure in both group II and III. HR was continuously increased although blood pressure already returned to normal

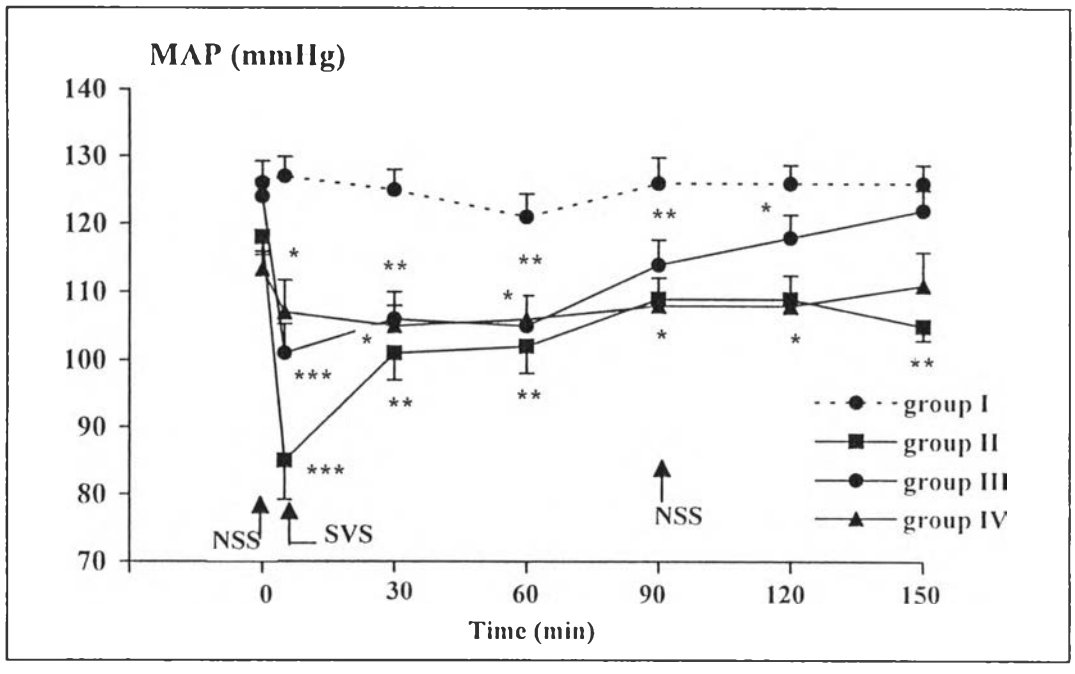

Figure 4.1 The alteration of mean arterial blood pressure (MAP) in response to SVS infusion. All values are mean±SEM. Statistical significant differences are indicated by *P<0.05 **P<0.01,***P<0.001. group I = control, group II, III, IV = SVS infusion of 200,150 and 100 mg/kg.BW. respectively.

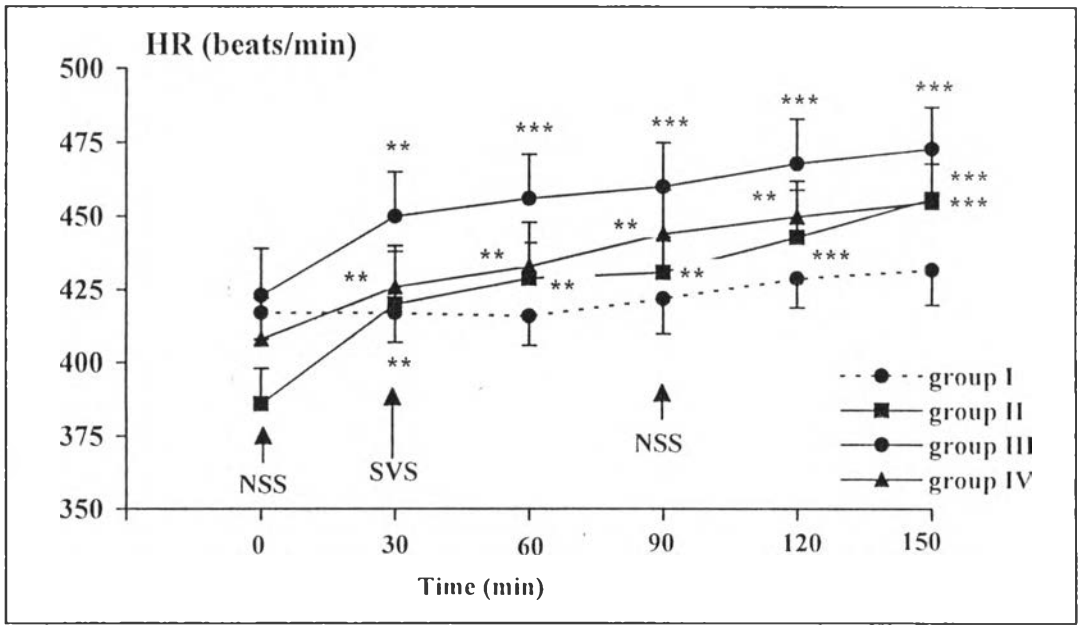

Figure 4.2 The alteration of heart rate in response to SVS infusion. All values are mean±SEM. Statistical significant differences are indicated by *P<0.05 **P<0.01,***P<0.001. group I = control, group II, III, IV = SVS infusion of 200,150 and 100 mg/kg.BW. respectively.

level during recovery period in group IV. HR was slightly but significantly elevated from 421±4 to 434±5 beats/min ($P<0.05$) in rats subjected to SVS intubation.

Changes of renal function in response to SVS administration

The alteration of effective renal plasma flow (ERPF) and effective renal blood flow (ERBF) is presented in table 4.1. During the first period of SVS infuison, ERPF was significantly increased from 17.01±2.46 to 24.65±3.08 ($P<0.05$), from 18.77±2.61 to 24.65±3.39 ($P<0.05$), and from 12.85±1.60 to 15.95±2.28 ($P<0.05$) ml/min/kg.BW in group II, III and IV respectively. Similarly, ERBF was enhanced from 27.18±3.88 to 44.32±6.44 ($P<0.05$), from37.12±4.87 to 48.80±6.34 ($P<0.05$), and from 23.36± 2.74 to 28.26±3.61 ($P<0.05$)ml/min/kg.BW in group II,III and IV respectively. The elevation of ERBF was taken place following the first period of SVS infusion in group II and III. SVS feeding did not have any influence on both ERPF and ERBF (table 4.7).

Although the first 30-min of SVS infusion produced the increase of ERBF, it did not affect on glomerular filtration rate (GFR) (table 4.1 and figure 4.3) in all groups. At the highest dose of SVS infusion (group II), GFR tended to be diminished but not statistical significant difference from control during the second period of SVS infusion. GFR significantly decreased from 5.92±0.67 to 3.89±0.70 ($P<0.05$) and 3.65±0.33 ($P<0.05$) during the first and second period of recovery. GFR was also reduced in group III but to a lesser extent comparing to group II during recovery period (table 4.1 and figure 4.3). In group IV, in contrast, GFR was unaffected

Table 4.1 Changes of renal hemodynamics in response to SVS infusion. Data are reported as mean±SEM. Abbreviations : ERBF = effective renal blood flow, GFR = Glomerular filtration rate, FF = Filtration fraction, group I = control, group II = SVS 200 mg/kg.BW, group III = SVS 150 mg/kg.BW, group IV = SVS 100 mg/kg.BW. Significant differences comparing to control period are indicated by *P<0.05, **P<0.01, ***P<0.001. The number of rats in each group is 8.

Periods / Parameters	control	SVS infusion		recovery	
	30 min	60 min	90 min	120 min	150 min
ERPF (ml/min/kg.BW)					
group I (control)	23.72±1.76	21.26±1.7	22.28±1.94	21.70±1.98	21.84±2.46
group II (SVS 200 mg/kg.BW)	17.01±2.46	24.65±3.08*	15.33±1.91	14.96±2.00	15.08±2.20
group III (SVS 150 mg/kg.BW)	18.77±2.61	24.65±3.39*	19.01±4.38	16.84±2.34	17.30±2.33
group IV (SVS 100 mg/kg.BW)	12.85±1.60	15.95±2.28*	13.78±1.14	14.33±1.88	15.01±1.76
ERBF (ml/min/kg.BW)					
group I (control)	43.23±3.05	38.67±2.30	39.32±3.22	38.03±3.45	38.19±4.37
group II (SVS 200 mg/kg.BW)	27.18±3.88	44.32±6.44*	27.93±3.42	26.70±3.56	26.14±3.73
group III (SVS 150 mg/kg.BW)	37.12±4.87	48.80±6.34*	36.06±8.69	31.13±4.36	32.12±4.20
group IV (SVS 100 mg/kg.BW)	23.36±2.74	28.26±3.61*	24.80±3.08	25.64±3.28	25.48±2.75
GFR (ml/min/kg.BW)					
group I (control)	6.02±0.31	5.69±0.29	5.70±0.45	5.47±0.24	5.70±0.45
group II (SVS 200 mg/kg.BW)	5.92±0.67	5.29±0.67	4.61±0.47	3.89±0.70*	3.65±0.33*
group III (SVS 150 mg/kg.BW)	5.97±0.59	5.31±0.91	5.14±0.92	4.81±0.82*	4.89±0.82*
group IV (SVS 100 mg/kg.BW)	5.02±0.49	5.54±0.47	5.27±0.37	4.64±0.34	5.42±0.40
FF (%)					
group I (control)	26.3±2.1	28.7±3.9	26.2±1.6	26.5±2.4	27.3±2.3
group II (SVS 200 mg/kg.BW)	40.2±7.8	25.9±5.8**	34.1±5.3	27.3±4.9	24.6±3.2
group III (SVS 150 mg/kg.BW)	34.0±3.0	22.3±3.3**	33.3±9.3	28.2±4.1	28.5±3.6
group IV (SVS 100 mg/kg.BW)	40.7±3.8	38.1±3.8	39.8±3.4	35.3±3.8	37.3±3.7

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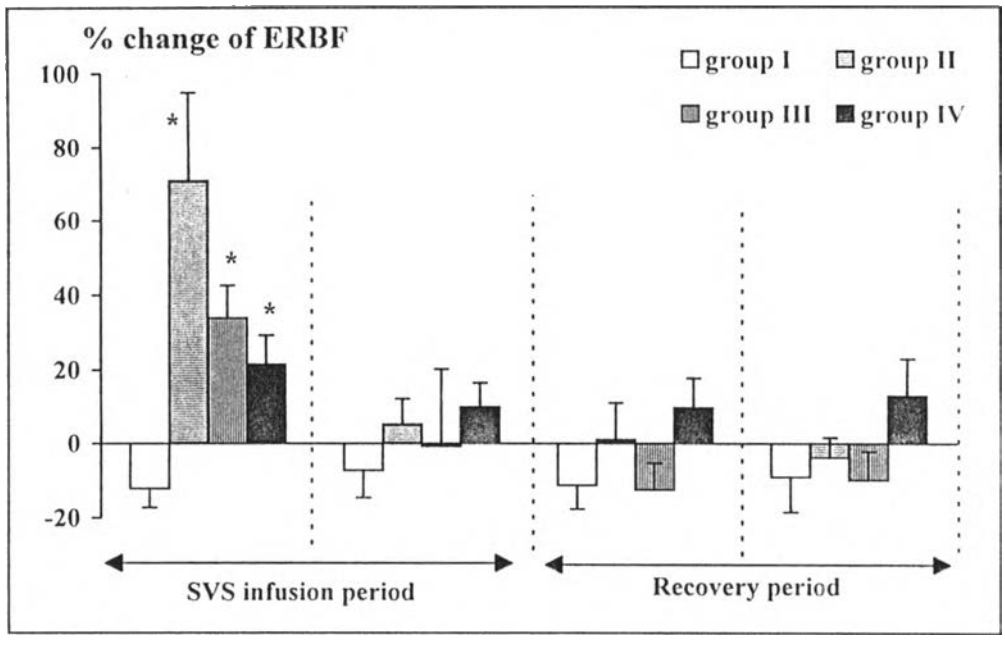

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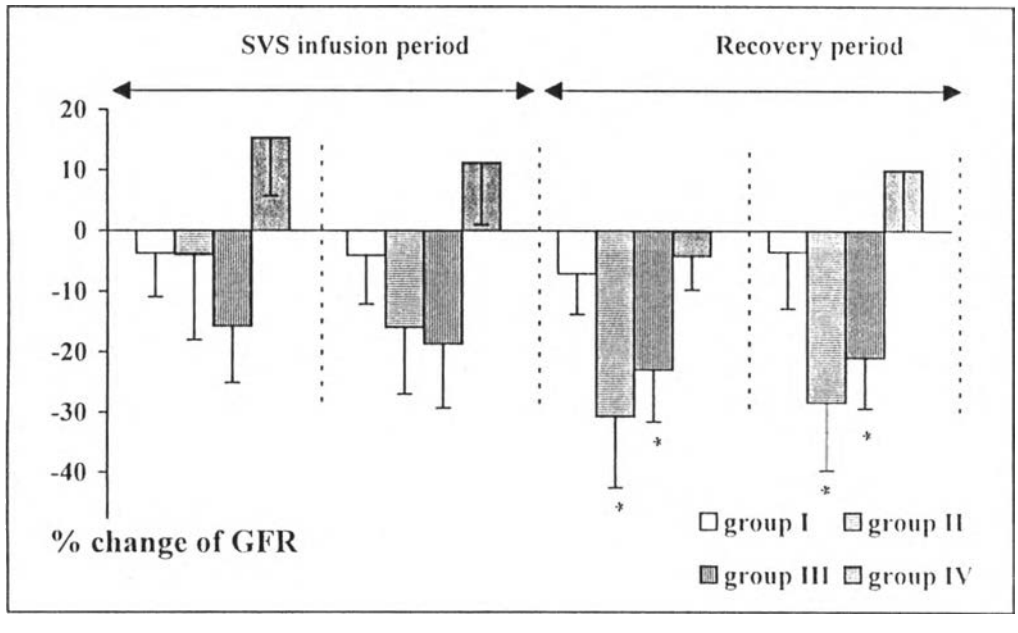


Figure 4.3 Percent change of effective renal blood flow(ERBF) (A) and glomerular filtration rate (GFR) (B) during and afterSVS infusion. Statistical significant differeces comparing control period are expressed as *P<0.05. group I (control), group II,III and IV = SVS infusion of 200,150 and 100 mg/kg.BW respectively.

throughout the experiment. GFR was remained unchanged after SVS intubation (5.63±0.23 versus 5.21±0.30 ml/min/kg.BW).

An augmentation of ERPF without change of GFR resulted in the reduction of filtration fraction (FF) from 40.2±7.8 to 25.9±5.8% ($P<0.01$) and from 34.0±3.0 to 22.3±3.3 %($P<0.01$) during the first period of SVS infusion in group II and III respectively. In group IV, FF was reduced but not statistical significant difference from the control period. FF was still decreased despite stopping SVS infusion in group II and III whereas no alteration of FF throughout the experiment in group IV. SVS intubation had no effect on FF.

Urine flow rate (V) was unaltered throughout the experiment in control group (table 4.2). Urine flow rate was markedly increased from 23.80±2.48 to 39.62±6.82 μl/min in group II($P<0.05$), from 22.85±2.35 to 34.43±3.61 μl/min in group III ($P<0.05$), and from 24.52±3.79 to 34.59±2.83 ($P<0.05$) μl/min in group IV in response to the first 30-min of SVS infusion. Similar result of urine flow rate was achieved in group of SVS feeding (from 26.73±3.00 to 33±2.0 μl/min, $P<0.05$). Urine flow was not significantly different from the control level following the latter period. The fractional excretion of urine (V/GFR) was significantly increased during the first period of SVS infusion in all groups. It was continuously raised until the first period of recovery in both group II and III ($P<0.05$) whereas no different response was observed from control period after the first 30-min of SVS infusion in group IV. Significant elevation of V/GFR was shown in response to SVS intubation (1.64±0.18 to 2.38±0.22%, $P<0.05$) (table 4.7).

Table 4.2 The alteration of urine flow (V) and fractional excretion of urine (V/GFR). Results are presented as mean±SEM. The experimental groups are the same as table 4.1. Significant difference values are shown by *P<0.05, **P<0.01. The number of rats in each group is 8.

Parameters \ Periods	control	SVS infusion		recovery	
	30 min	60 min	90 min	120 min	150 min
V (μl/min)					
group I (control)	21.26±3.43	18.98±2.34	24.48±3.90	29.80±5.12	30.78±4.86
group II (SVS 200 mg/kg.BW)	23.80±2.48	39.62±6.82*	27.37±2.82	25.79±3.10	22.63±3.39
group III (SVS 150 mg/kg.BW)	22.85±2.35	34.43±3.61*	25.11±4.00	24.05±3.27	25.41±2.70
group IV (SVS 100 mg/kg.BW)	24.52±3.79	34.59±2.83*	30.59±4.24	26.47±4.69	30.80±6.01
V/GFR (%)					
group I (control)	1.29±0.21	1.23±0.18	1.57±0.26	1.99±0.33	1.94±0.25
group II (SVS 200 mg/kg.BW)	1.64±0.22	2.90±0.43**	2.44±0.40*	3.01±0.52*	2.76±0.54
group III (SVS 150 mg/kg.BW)	1.57±0.29	2.99±0.58**	2.83±0.91	2.53±0.65*	2.57±0.62
group IV (SVS 100 mg/kg.BW)	1.91±0.26	2.44±0.26*	2.17±0.28	2.13±0.34	2.14±0.33

The change of urinary electrolyte excretion in response to SVS administration

The value of plasma electrolyte composition (Na^+,K^+, Cl^-) is presented in table 4.3. Plasma concentration of Na^+, K^+ and Cl^- was remained unaltered throughout the experimental period in control group. No significant changes of plasma electrolytes concentration during the first period of SVS infusion were observed. On the other hand, they tended to be raised in the latter period of experiment but not statistical significant differences from the control period. In contrast, plasma electrolyte concentration was significantly high from 139.42±1.28 to 143.48±0.85 mEq/L for Na^+, 3.62±0.05 to 3.79±0.07 mEq/L for K^+, and 110.73±1.37 to 116.67±1.71 mEq/L for Cl^- ($P<0.05$) in rats given of SVS intubation (table 4.7)

The urinary excretion of Na^+, ($U_{Na}V$) was markedly high from 11.18±1.75 to 20.27±2.97μEq/min/kg.BW($P<0.01$),from 11.07±1.79 to 16.61±3.85 μEq/min/kg.BW and from 15.32±1.93 to 20.16±2.61 ($P<0.05$) μEq/min/kg.BW during the first period of SVS infusion in group II, III and IV respectively. Similarly, urinary excretion of Cl^- ($U_{Cl}V$) was significantly raised from 12.43±1.72 to 22.89±2.66 μEq/min/kg.BW ($P<0.01$), 11.05±1.80 to 17.23±3.02 μEq/min/kg.BW ($P<0.05$), and 10.39±1.47 to 16.13±2.00 ($P<0.05$) μEq/min/kg.BW for group II, III and IV respectively. Urinary excretion of K^+ (U_KV) was not statistical significant difference from the control value. $U_{Na}V$ was remained high in the second period of SVS infusion in group II (16.65± 2.55, $P<0.05$) whereas no significant change in both group III and IV. Urinary electrolytes excretion did not alter in the rest of experiment in all groups. In the group of SVS feeding, there were significant rise of $U_{Na}V$ from 9.14±1.18 to 14.16± 1.03

Table 4.3 Effect of SVS infusion on plasma electrolytes concentration. All results are shown as mean±SEM. Abbreviations : P_{Na} P_K, P_{Cl} = plasma concentration of sodium, potassium and chloride respectively. The experimental groups are similar to table 4.1. Significant differences comparing to control period are indicated by *P<0.05, **P<0.01. The number of rats in each group is 8.

Periods / Parameters	control	SVS infusion		recovery	
	30 min	60 min	90 min	120 min	150 min
P_{Na} (mEq/L)					
group I (control)	137.75±0.88	137.38±1.44	138.39±0.86	138.38±0.53	139.13±0.74
group II (SVS 200 mg/kg.BW)	136.50±1.09	136.88±1.23	138.00±0.14	138.23±1.09	139.34±1.28
group III (SVS 150 mg/kg.BW)	136.25±1.18	136.38±1.70	138.50±1.24	139.63±2.73	141.88±2.47*
group IV (SVS 100 mg/kg.BW)	138.75±1.18	140.25±0.80	139.88±1.20	143.50±2.41	140.25±2.77
P_K (mEq/L)					
group I (control)	3.43±0.14	3.40±0.17	3.40±0.13	3.38±0.10	3.41±0.13
group II (SVS 200 mg/kg.BW)	3.55±0.10	3.74±0.15	3.80±0.14	3.93±0.22	3.86±0.17
group III (SVS 150 mg/kg.BW)	3.56±0.07	3.40±0.14	3.73±0.14	3.96±0.13	3.81±0.14
group IV (SVS 100 mg/kg.BW)	3.80±0.12	3.84±0.13	3.84±0.10	3.94±0.11	3.95±0.10
P_{Cl} (mEq/L)					
group I (control)	101.25±1.13	102.25±1.08	105.13±1.56	104.63±1.76	103.25±1.56
group II (SVS 200 mg/kg.BW)	101.00±1.94	104.75±1.79	103.63±3.22	103.88±1.96	106.13±2.59
group III (SVS 150 mg/kg.BW)	106.38±1.73	108.50±1.70	105.38±1.31	106.13±1.44	108.63±1.56
group IV (SVS 100 mg/kg.BW)	102.63±2.45	103.75±1.46	102.75±1.83	104.38±2.41	107.38±3.43

Table 4.4 The urinary excretion of electrolytes in response to SVS infusion. Results are presented as mean±SEM. Abbreviations : $U_{Na}V$, U_KV, $U_{Cl}V$ = urinary excretion of sodium, potassium and chloride respectively. The experimental groups were the same as table 4.1. Signifiant differences comparing to control period are indicated by *P<0.05, **P<0.01. The number of rats in each group is 8.

Periods / Parameters	control	SVS infusion		recovery	
	30 min	60 min	90 min	120 min	150 min
$U_{Na}V$ (μEq/min/kg.BW)					
group I (control)	11.16±2.84	9.62±1.06	12.39±1.75	13.78±1.73	14.32±1.67
group II (SVS 200 mg/kg.BW)	11.18±1.75	20.27±2.97**	16.65±2.55*	14.98±2.26	10.16±2.37
group III (SVS 150 mg/kg.BW)	11.07±1.79	16.61±3.85	11.04±1.82	10.45±2.15	12.47±3.23
group IV (SVS 100 mg/kg.BW)	15.32±1.93	20.16±2.61*	19.48±3.15	15.81±2.28	17.02±3.08
U_KV (μEq/min/kg.BW)					
group I (control)	8.39±1.21	6.92±0.71	7.30±0.84	7.05±0.81	6.41±0.80
group II (SVS 200 mg/kg.BW)	6.46±0.76	7.72±0.53	6.15±0.52	5.09±0.84	5.28±0.80
group III (SVS 150 mg/kg.BW)	6.94±1.35	7.15±1.33	5.88±1.47	4.79±1.01*	5.60±0.91
group IV (SVS 100 mg/kg.BW)	6.46±0.80	7.77±0.62	7.16±1.00	6.11±0.72	7.18±1.13
$U_{Cl}V$ (μEq/min/kg.BW)					
group I (control)	11.81±1.67	10.83±1.36	12.63±1.81	13.96±1.55	14.83±1.44
group II (SVS 200 mg/kg.BW)	12.43±1.72	22.89±2.66**	15.72±1.91	14.60±3.36	11.39±1.73
group III (SVS 150 mg/kg.BW)	11.05±1.80	17.23±3.02*	11.27±2.74	10.65±1.81	12.72±1.73
group IV (SVS 100 mg/kg.BW)	10.39±1.47	16.13±2.00*	13.49±2.70	12.12±2.67	12.69±2.33

μEq/min/ kg.BW ($P<0.01$) and for $U_{Cl}V$ from 14.31±1.32 to 18.67±1.30 μEq/min/ kg.BW ($P<0.05$). The U_KV was slightly increased but not significant difference from the control group.

Urinary and fractional excretion of electrolytes were constant throughout the experimental periods in the control group (table 4.4-4.5). The first 30-min of SVS infusion enhanced fractional excretion of Na (FE_{Na}) in the dose dependent manner (table 4.4 and figure 4.4). FE_{Na} was increased from 1.58±0.29 to 2.95±0.46 % ($P<0.001$), 1.49±0.29 to 2.66±0.59 %($P<0.01$),and 2.21±0.18 to 2.61±0.27%($P<0.05$) in group II, III and IV respectively. FE_{Na} was remained in the latter and recovery periods in group II and III. FE_{Na} of group IV was significantly increased in the latter period of SVS infusion. The same response as FE_{Na} was displayed in fractional excretion of chloride (FE_{Cl}). Fractional excretion of K^+ (FE_K) was slightly but significantly raised from 32.35±3.70 to 41.68±3.06 % ($P<0.05$) and 30.66±3.40 to 39.09±2.47% ($P<0.05$) in group II and III respectively. During the second period of SVS infusion, FE_K slightly high and returned back to normal value in both group after stopping SVS administration. In contrary, no significant change of FE_K appeared throughout experiment in group IV. The elevation of FE_{Na}, FE_K and FE_{Cl} was also taken place ($P<0.05$) in rats treated with SVS intubation.

Plasma osmolality (Posm), osmolar clearance (Cosm) and free water clearance ($C H_2O$) are shown in table 4.6. Both group II and III , Posm slightly increased but not statistical significant difference from control period during SVS infusion. Significant rise of Posm was apparent in the last period of recovery in both group II and III.

Table 4.5 The alterations of fractional excretion of electrolytes in response to SVS infusion. Values are mean±SEM. Abbreviations : FE_{Na}, FE_K, FE_{Cl} = fractional excretion of sodium, potassium and chloride respectively. The experimental groups are the same as table 4.1. Significant differences comparing to control period are indicated by *P<0.05, **P<0.01. The number of rats in each group is 8.

Parameters \ Periods	control	SVS infusion		recovery	
	30 min	60 min	90 min	120 min	150 min
FE_{Na} (%)					
group I (control)	1.36±0.32	1.28±0.18	1.59±0.19	1.85±0.23	1.81±0.17
group II (SVS 200 mg/kg.BW)	1.58±0.29	2.95±0.46***	2.85±0.58**	3.39±0.67**	2.25±0.49*
group III (SVS 150 mg/kg.BW)	1.49±0.29	2.66±0.59**	1.94±0.40*	1.86±0.35*	2.45±0.81
group IV (SVS 100 mg/kg.BW)	2.21±0.18	2.61±0.27*	2.64±0.38	2.32±0.25	2.52±0.50
FE_K (%)					
group I (control)	42.59±8.53	37.08±4.75	38.88±4.86	38.50±4.35	32.95±3.04
group II (SVS 200 mg/kg.BW)	32.35±3.70	41.68±3.06*	36.71±3.06*	35.59±3.68	41.48±5.10
group III (SVS 150 mg/kg.BW)	30.66±3.40	39.09±2.47*	32.01±4.87	26.63±4.32	32.86±5.35
group IV (SVS 100 mg/kg.BW)	36.96±6.44	37.95±3.49	36.95±6.20	33.84±3.63	36.56±6.30
FE_{Cl} (%)					
group I (control)	1.98±0.28	1.88±0.26	2.16±0.34	2.44±0.26	2.51±0.18
group II (SVS 200 mg/kg.BW)	2.21±0.32	4.38±0.49***	3.61±0.56**	4.05±0.71*	3.28±0.44*
group III (SVS 150 mg/kg.BW)	1.75±0.21	3.30±0.53**	2.30±0.39*	2.42±0.39*	2.78±0.55
group IV (SVS 100 mg/kg.BW)	2.24±0.40	3.05±0.56*	2.63±0.43	2.61±0.43	2.47±0.34

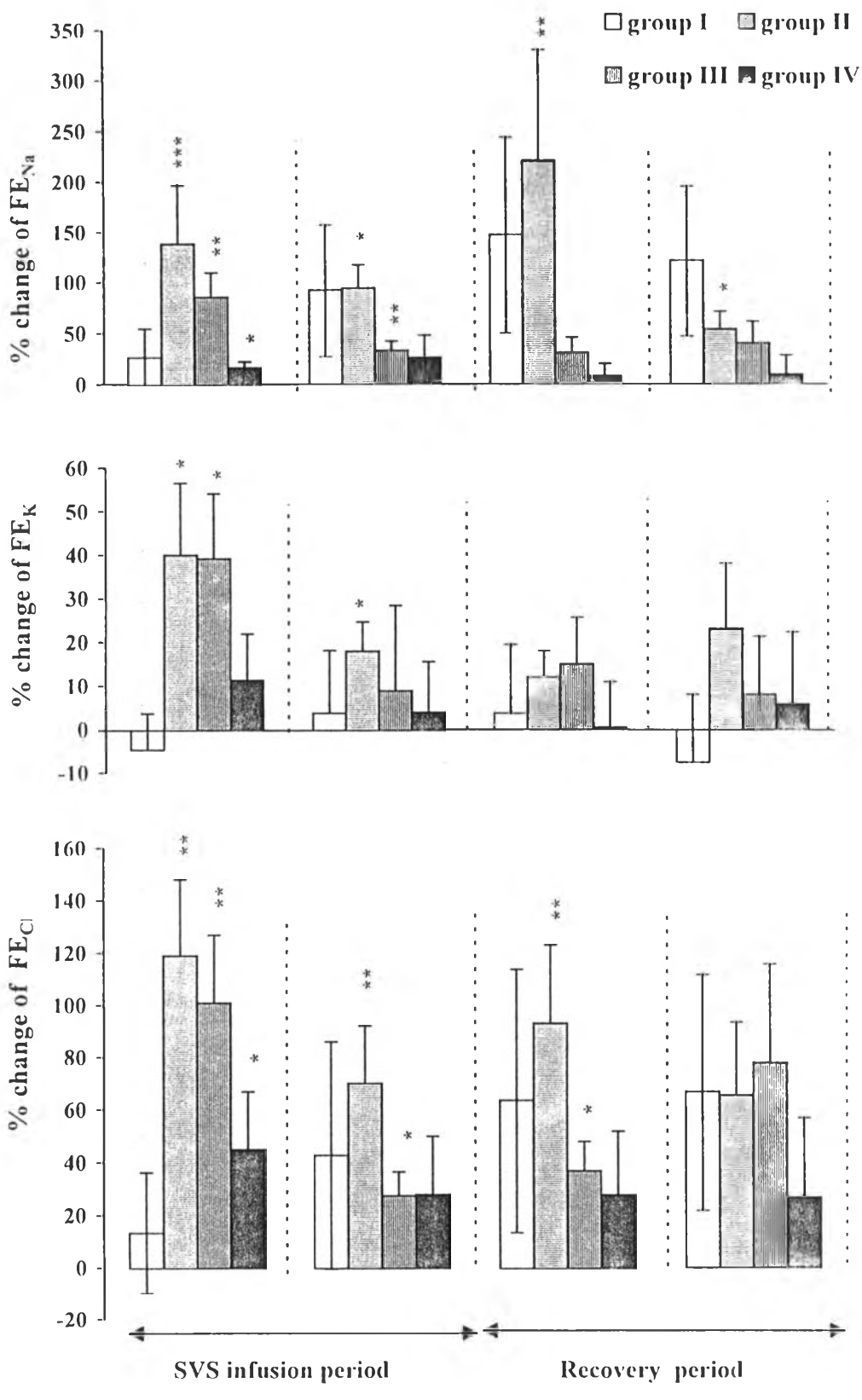


Figure 4.4 Percent changes of fractional excretion of Na^+,K^+,and Cl^- in rat treated with SVS infusion at the dose of 200 (group II),150 (group III) and 100 (group IV) mg/kg.BW. Significant differences are indicated by *P,0.05, **P<0.01, ***P<0.001.

Table 4. 6 The effect of SVS infusion on plasma osmolality (Posm), osmolar clearance (Cosm) and free water clearance (CH_2O). All values are presented as mean±SEM. The significant differences comparing to control period are indicated by *P<0.05, **P<0.01. The number of rats in each group is 8.

Parameters \ Periods	control	SVS infusion		recovery	
	30 min	60 min	90 min	120 min	150 min
Posm (mOsm/L)					
group I (control)	287.51±1.94	286.04±1.94	287.81±2.10	287.91±1.61	288.09±1.11
group II (SVS 200 mg/kg.BW)	289.23±2.39	293.33±2.68	296.98±3.15*	298.03±3.00	299.66±4.00*
group III (SVS 150 mg/kg.BW)	287.39±3.80	292.17±5.63	294.28±7.86	295.63±6.31	297.03±4.62*
group IV (SVS 100 mg/kg.BW)	288.69±2.25	294.97±2.41*	293.58±3.08	293.63±2.22	282.88±2.36
Cosm (μL/min)					
group I (control)	69.64±7.65	62.90±4.78	64.12±6.26	71.65±7.98	70.32±6.71
group II (SVS 200 mg/kg.BW)	60.27±6.62	95.53±20.91*	68.83±13.45	60.78±10.56	52.04±6.77
group III (SVS 150 mg/kg.BW)	65.03±9.93	82.19±8.86	63.80±7.42	55.24±8.49	59.84±7.70
group IV (SVS 100 mg/kg.BW)	62.75±6.82	70.51±7.56*	71.03±10.80	68.26±9.07	58.90±7.62
CH_2O (μL/min)					
group I (control)	-48.38±5.05	-43.92±3.56	-39.65±4.79	-41.85±3.94	-39.53±3.12
group II (SVS 200 mg/kg.BW)	-36.48±5.33	-55.91±14.61	-41.46±11.38	-34.98±8.83	-29.41±5.78
group III (SVS 150 mg/kg.BW)	-42.18±9.46	-47.92±6.22	-38.69±5.67	-31.20±6.30	-34.43±5.52
group IV (SVS 100 mg/kg.BW)	-38.23±7.99	-35.90±7.01	-40.44±9.03	-41.80±5.65	-28.10±7.97

Cosm was increased from 60.27±6.62 to 95.53±20.91μL/min in group II($P<0.05$), from 65.03±9.93 to 82.19±8.86 μL/min in group III, and from 62.75±6.82 to 70.51± 7.56 μL/min in group IV($P<0.05$) in response to the first period of SVS infusion. Beyon this period, Cosm was unaltered. CH_2O was not changed throughout the experiment in control group. It tended to be reduced (more negative) but not significant different from control period in response to the first period of SVS infusion in both group II and III. No significant changes of CH_2O was noted beyond this period. CH_2O was likely to be unchanged throughout the experimental period in group IV. SVS feeding resulted a significant rise of Posm from 289.84±2.67 to 297.54±1.63 mOsm/L ($P<0.05$) (table 4.7). Similarly, Cosm was raised from 62±4 to 74±3 μL/min ($P<0.05$). The reduction of CH_2O was induced but not statistical significant difference from -36.1±2.6 in control group to -41.3±2.2 μL/min in SVS intubation group.

Histopathological changes of renal tissues after given of SVS

Fig 4.5-4.8 show the general appearance of renal tissue under light microscope in control and SVS-treated rat. Renal tissue of the control rat showed the normal appearance. In contrast, general tubular degeneration including proximal tubular cells was produced after SVS infusion. There were hydropic (vacuolar) degeneration in cytoplasm and cloudy swelling. Most distal tubular lumen were filled with protenacious materials and cells debris. Glomeruli and general vessels lining along tubular cells were congestion especially in rats subjected to the highest concentration of SVS. The higher the SVS administration, the larger the damage of renal tubular

Table 4.7 Alterations of general circulation and renal function after 6 hours of SVS intubation. Results are shown as mean±S.E.M. Abbreviations are the same as table 4.1-4.6. Significant difference comparing between control and SVS intubation group areindicated by *P<0.05, **P<0.01, ***P<0.001.The number of rats in each group is 9.

Group / Parameters	Control	SVS intubation
MAP(mmHg)	119±2	115±1*
HR (beats/min)	421±4.39	434±4.68*
V (μL/min)	25.73±2.6	33.00±3.40*
GFR (ml/min/kgBW)	5.63±0.23	5.21±0.30
V/GFR (%)	1.64±0.18	2.38±0.22**
ERPF (ml/min/kgBW)	19.72±0.62	20.58±0.94
ERBF (ml/min/kgBW)	38.50±1.10	39.60±1.82
FF (%)	29.13±1.42	25.95±1.71
P_{Na} (mEq/L)	139.42±1.28	143.48±0.85*
P_K (mEq/L)	3.62±0.05	3.79±0.07*
P_{Cl} (mEq/L)	110.73±1.37	116.67±1.71**
FE_{Na} (%)	1.25±0.18	2.05±0.21**
FE_K (%)	35.16±3.58	45.87±2.93*
FE_{Cl} (%)	2.46±0.29	3.34±0.31*
$U_{Na}V$ (μEq/min/kg BW)	9.14±1.18	14.16±1.03**
U_KV (μEq/min/kg BW)	7.47±0.30	8.50±0.44*
$U_{Cl}V$ (μEq/min/kg BW)	14.31±1.32	18.67±1.29*
P_{osm} (mOsm)	289.84±2.67	297.54±1.63*
C_{osm} (μL/min)	62±4	74±3*
C H_2O (μL/min)	-36±2.6	-41±2.6

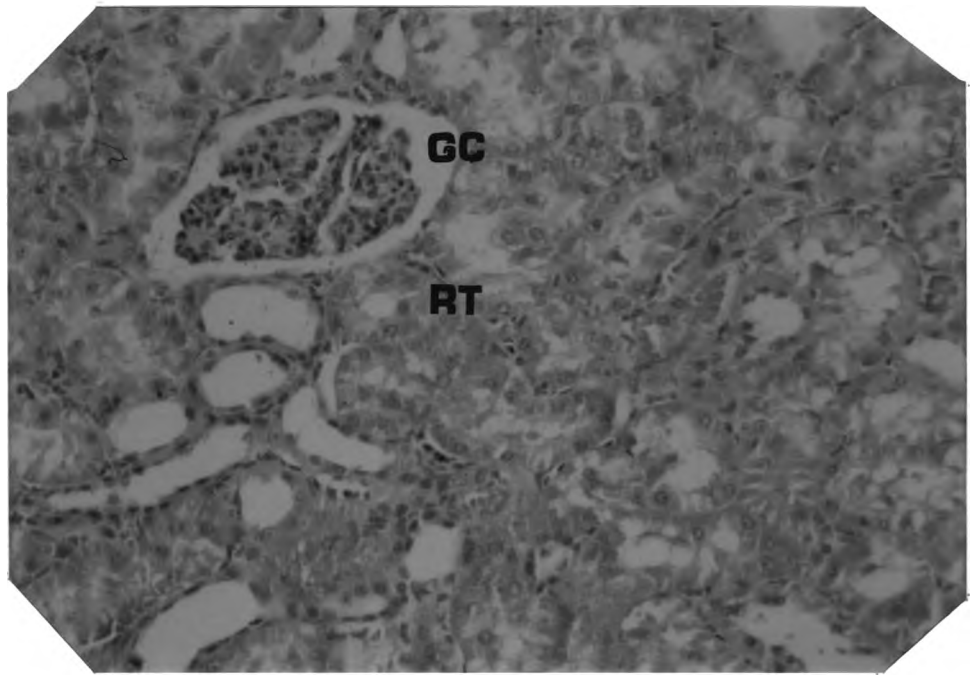
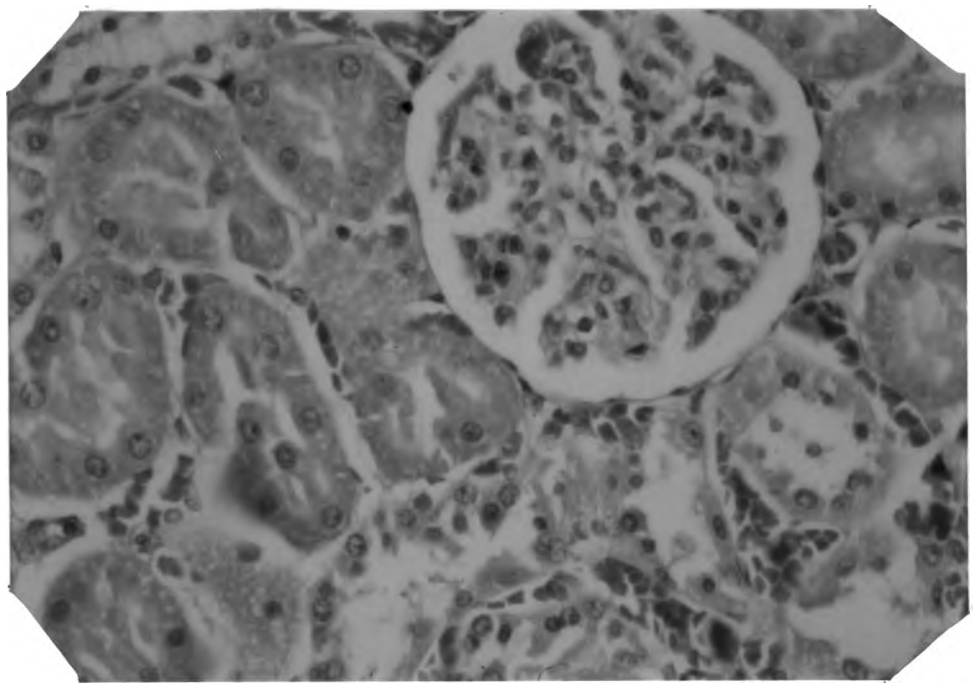


Figure 4.5 Light micrographs of renal tissue of rat given normal saline with the rate 1%kg.BW/hr illustrating the normal appearance of both glomerular capillary and renal tubular cell (X200). The tissue was stained with hematoxylin and eosin. (GC= glomerular capillaries, RT= renal tubule)

A



B

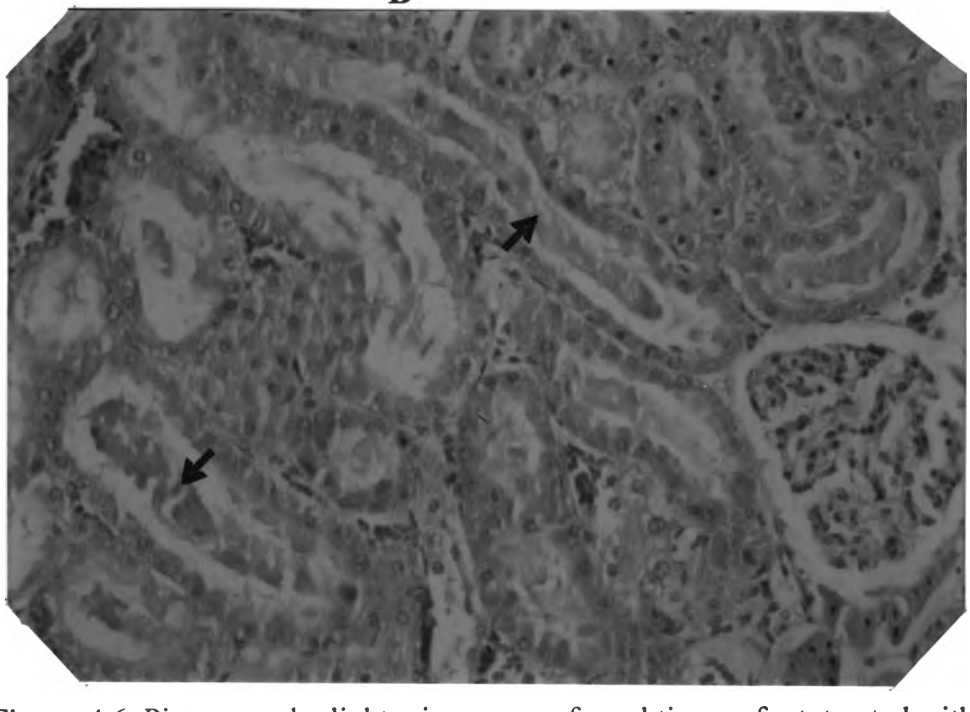


Figure 4.6 Picture under light microscope of renal tissue of rat treated with SVS infusion(200 mg/kg.BW)showing diffused congestion including glomerular capillaries but not hemorrhage (A), tubular cell degeneration with hydropic vacuole in cytoplasm (B). Renal tubular lumen in distal tubule are filled with proteinacious materials and cell debris as indicated by the arrows (B). (AX400, BX200) . The tissue was stained with hematoxylin and eosin.

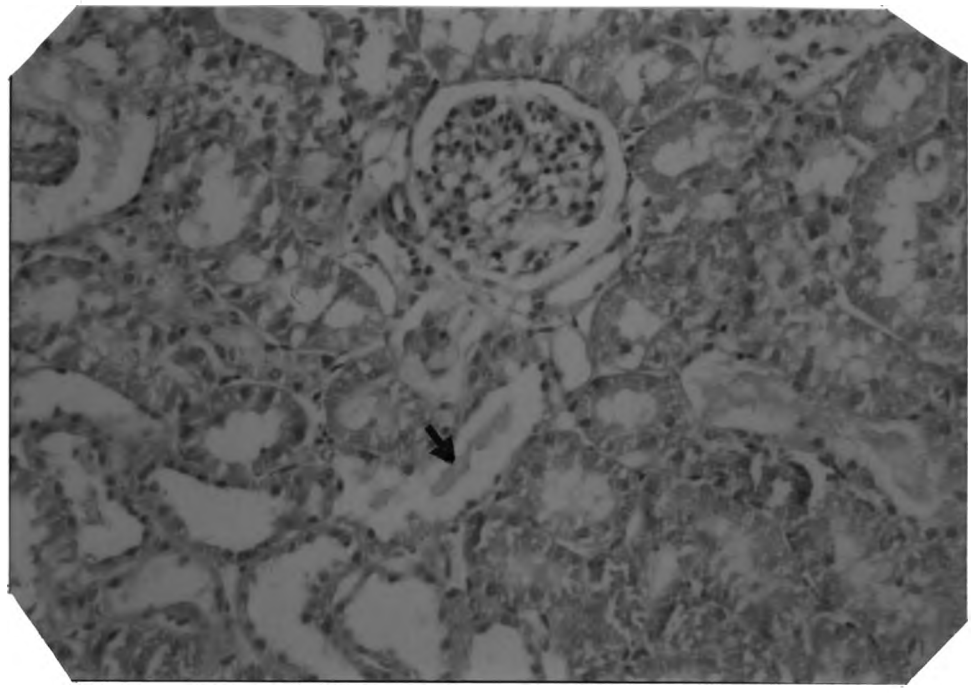


Figure 4.7 Light micrographs of renal tissue of rat treated with SVS infusion (150 mg/ kg.BW). There are diffused congestion including glomerular capillaries Renal tubular cell degeneration with hydropic vacuole is appeared. Slightly proteinacious materials and cell debris as indicated by the arrows is also detected in distal tubular lumen. The tissue was stained with hematoxylin and eosin. (X200) .

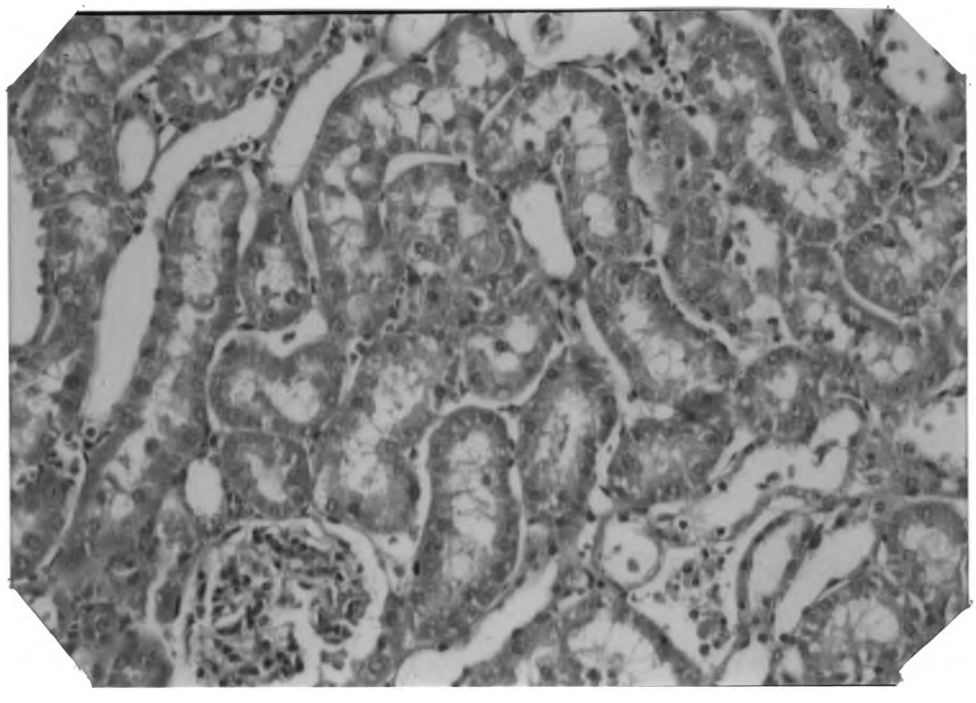


Figure 4.8 The picture under light microscope of rat subjected to SVS infusion (100 mg/kg.BW). There is cloudy swelling but no hydropic vacuole in renal tubular cell. No proteincious materials is apparent in tubular lumen. The tissue was stained with hematoxylin and eosin. (X200)

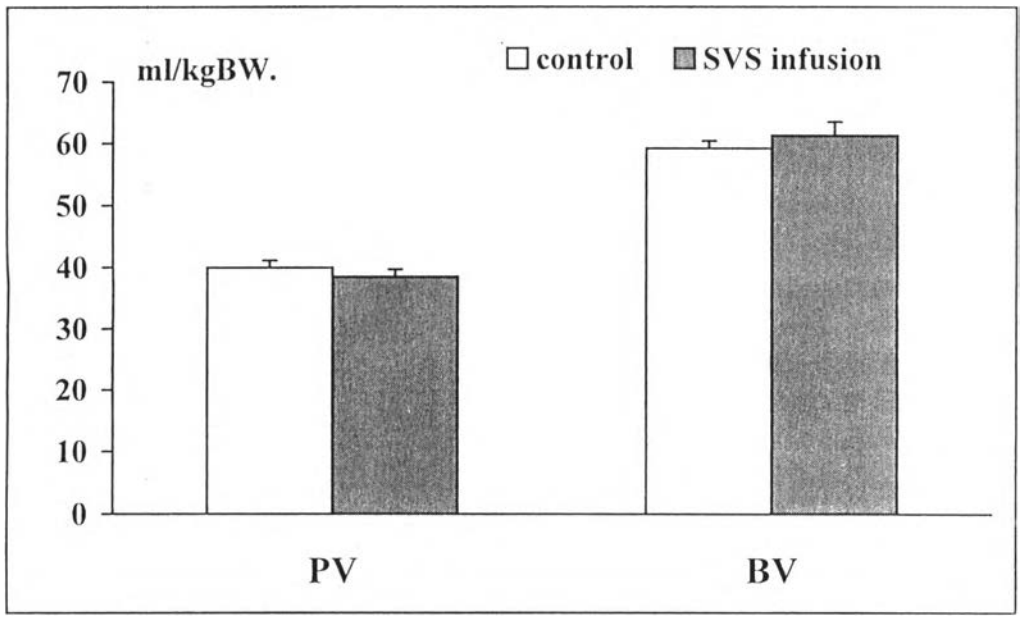


Figure 4.9 The alteration of plasma volume (PV) and blood volume (BV) in control and SVS infusion group. The values were presented as mean±SEM. There are no significant differences of both plasma volume and blood volume between control and SVS infusion group.

cells. Slightly changes were apparent in both glomeruli and renal tubular cells of rat given lowest dose of SVS infusion (group IV) and SVS intubation.

Changes of plasma volume and blood volume during SVS infusion

Plasma volume (PV) and blood volume (BV) were measured in rats treated with SVS infusion (figure 4.9). Plasma volume and blood volume was 40.0±0.9 ml/kg.BW and 59.3±1.2 ml/kg.BW in control rats. No significant change of plasma volume (38.5±1.3 ml/kg.BW) and blood volume (61.3±2.0 ml/kg.BW) was noted during SVS infusion. It was interesting that during SVS infusion, blue-skin colour was seen throughout the body within 8-10 minutes following Evan's blue dye injection ,and remained appeared throughout the experiment whereas it did not occur in control group.

Discussion

The present study indicates the effect of SVS both orally and intravenously on general circulation and kidney function. Intravenous infusion of SVS lowered blood pressure in the dose dependent manner. Similar results were observed by Melis and Sainati (1991 a,b) and Kurahashi et al. (1982). Melis (1992b) has also reported the hypotensive effect of SVS in Goldblalt 2-kidney/1-clip hypertensive rats. The possible mechanisms responsible to its hypotensive effect are still unclear. Melis and Sainati (1991a) reported that the effect of SVS infusion on renal fuction was partly dependent on prostaglandin.

Blood pressure was gradually increased after the transitory decrease at lowest level of blood pressure which was quite similar to endotoxin treated rats (Schaller et al., 1985 ; Preiser et al., 1994). This may be the result from sympathetic compensatory response to hypotension that could be seen by the elevation of HR in the present experiment. Although blood pressure was improved but still lower than that of control period in both group II and III. The same response was also reported in mice treated with 0.49 g/kg.BW of SVS intravenously (Kurahashi et al., 1982). Despite the termination of SVS infusion, blood pressure was uncompletely recovered except that of group subjected to the lowest dose of SVS infusion (group IV). The reduction of blood pressure in latter period of experiment was not the timed effect because no changes of blood pressure occured in control group throughout experiment. The possible reason may be that SVS is incompletely excreted out of the body. This possibility was supported by the results of Ishü and Bracht (1995) that the concentration of SVS remained constant after 2 hours of SVS infusion into isolated rat liver. Furthermore, the possible formation to other products, especially steviol, was investigated chromatographically with negative result (Ishü and Bracht ,1995). Though liver is known to be the major organ to metabolize several substances, the other organs like kidney also play a role. SVS was found to be excreted in urine (Melis, 1992a). However, SVS was not totally excreted within 1 hour (Nakayama et al.,1986). Therefore, SVS's effect should still exist despite SVS infusion was not allowed. Six hours after SVS feeding caused a slight but significant reduction of blood pressure which implies that the effect of SVS on hypotension appeared not only intravenous infusion but also oral administration. Chronic administration of aqueous

extract of Stevia rebaudiana (40-60days) also induced hypotension (Melis, 1995). The depression of blood pressure may be the result from the vasodilatation and/or the reduction of plasma volume. Because SVS infusion was shown to induce diuresis as mention earlier (table 4.2). General congestion and degeneration of several organs were also demonstrated (Glinsukon et al, 1988a). These indicate that the distribution of fluid from intravascular compartment to the other part may be possible. However, no significant alteration of plasma and blood volume were noted in response to SVS infusion (figure 4.9). Therefore, the hypotensive effect of SVS is likely due to the peripheral vasodilatation. Supporting the interpretation for the peripheral vasodilatation is the appearance of general blue-skin colour after Evan's blue dye injection which was not observed in the control rat.

SVS infusion raised HR which was corresponding to the depression of blood pressure. Tachycardia was remained throughout the experiment in all groups of SVS infusion. The elevation of HR may be the sympathetic response to the hypotension (Bullock,1995). It was found that plasma norepinephrine and epinephrine were raised in rats treated with endotoxin-induced hypotension (Schaller et al., 1985). However, the rise of HR persisted despite the normalization of blood pressure in the last period of the experiment in group III and during recovery period of group IV. It is not known the reason why HR was still high whereas blood pressure was reversd. Time effect was not to be the case even though HR tended to be raised in the last period of control group but the magnitude was not as much as that of SVS infusion group (figure 4.2). It was reported that SVS administration reduced the duration of electrical systole

(Humboldt and Boeckh, 1978). It is presumably that SVS has direct stimulation on the heart other than the effect on blood vessel.

The effect of SVS on renal hemodynamics were clearly shown during the first period of SVS infusion. Renal vasodilation without change in GFR resulting the reduction of FF was noted. This indicates that SVS induces renal vasodilatation both afferent and efferent arterioles. This agrees with the data obtained by Melis and Sainati (1991a,b). The renal hemodynamics response to SVS infusion was likely similar to the action of several vasodilators such as acetylcholine and bradykinin (Christine et al.,1976 ;Thomas et al.,1983). SVS intubation, in contrary, had no influence on renal hemodynamics. It is presumably that SVS ingestion was converted to steviol . It was reported that SVS was converted to be steviol after incubation in rat caecal microflora (Luckey, 1972; Wingard et al, 1980). However, there is no experimental evidence to support the action of steviol on general circulation. During recovery period, RBF was quite constant but GFR was lowered. Time effect did not contribute to these alterations because they were remained normal throughout the experiment in control group. The reduction of GFR was associated to the enhancement of Na^+ excretion. Tubuloglomerular feedback mechanism may be operated. That is, Na^+ delivery to macula densa might increase, and thereby feedbacks to constricts afferent arteriole(Vander,1995; Bullock,1995a). However, this possibility is unlikely since RPF was unchanged instead of reduced during recovery period. Some other mechanisms such as glomerular capillary abnormality such as glomerular capillary damage, may be expressed. Panichkul et al. (1988) found the rise of plasma urea nitrogen and creatinine in rats given of subcutaneous injection of SVS.

Furthermore, severe congestion in capillaries of both interstitium and glomeruli was observed(Glinsukon et al.,1988b). From our histopathological examination,glomerular congestion was clearly shown in rat given of highest dose of SVS (figure 4.6). The same appearance but lesser degree was detected in group III and IV respectively. From these experimental evidences, SVS infusion is likely induce glomerular capillaries congestion and thereby leading to the reduction of GFR.

Both intravenous infusion and intubation of SVS provoked diuresis and elevation of electrolyte excretion. These circumstances were pronounced during the first 30 min period of SVS infusion. The facilitation of electrolyte excretion without changes of GFR and plasma electrolytes during the first period of SVS infusion (table 4.1 and 4.3) may be explained by the depression of tubular reabsorption which presumably due to the result from 2 possible causes. First, the alteration of physical forces at peritubular capillary caused by the more maked elevation of renal plasma flow. The increase of hydrostatic pressure inside the peritubular capillaries produced by the elevation of RPF, reduces tubular Na^+ reabsorption.(Vander, 1995). Second, the direct effect of SVS on renal tubular reabsorptive function, for example, enzyme Na^+,K^+ATPase, renal mitochondrial function, the integrity of reabsorptive cells, etc (Gullans and Mandel, 1992). Proximal tubule is the major site for H_2O and electrolyte reabsorption. Several lines of evidences have suggested that proximal tubule was highly vulnerable to various nephrotoxic agents (Stonard, 1990). Toskulkao and his co-workers (1994) showed the significant elevation of alkaline phosphatase, γ-glutamyl transpeptidase and N-acetyl β-D-glucuronidase which were the markers for proximal tubular damages in rat subjected to subcutaneous injection of SVS for 9

hours. The degenerations of proximal convoluted tubule, distal tubule and collecting duct were also elicited in hamster treated with SVS (Glinsukon et al., 1988b). From these experimental results it seems to be that SVS exhibited its influence on proximal tubular Na^+ reabsorption. However, the mechanisms responsible to the interferance have not yet elucidated. The enzyme Na^+,K^+ ATPase which is the key enzyme for tubular Na^+ reabsorption may participate, and should be further clarified. However, other mechanisms such as hormonal changes in response to SVS administration should be also investigated.

Fractional urinary excretion of Na^+ and Cl^- remained increased in the second period of SVS infusion. The alteration of physical forces at peritubular capillaries but to a lesser extent were remained which may be responsible to NaCl excretion. However, the magnitude of change of physical forces was so little to produce natriuresis (table 4.1 and 4.5). Therefore, other mechanisms ,for example, direct tubular effect may be possible. Like the effect on blood pressure, fractional urinary excretion of Na^+ and Cl^- was continuously increased in group II during recovery period. It is presumably due to the sustaining effect of SVS which may not totally eliminated out of the body as outlined earlier. The abnormality of tubular cells was observed from histopathological examination after the end of experiment. It was not known when proximal tubular abnormality was produced. Therefore, the rise of electrolyte excretion during and after SVS infusion may be the result from both physical forces and/or direct influence of SVS infusion on tubular reabsorptive cells.

K^+ excretion is quite different from Na^+ and Cl^- excretion. While Na^+ and Cl^- excretion were markedly increased, K^+ excretion seemed to be slightly changed, especially after the first period of SVS infusion. Moreover, K^+ excretion was unaltered in group IV. In general, 50% and 40% of filtered K^+ is reabsorbed at proximal tubule and loop of Henle respectively (Vander,1995). However, most of K^+ appearing in urine is secreted by connecting and collecting tubule. It is more likely the reduction of proximal tubular Na^+ reabsorption during SVS infusion as previously suggested, decreases proximal tubular K^+ reabsorption (Vander,1995). Approximately 80-90% of filtered HCO_3 is resbsorbed at proximal tubule (Bullock,1995a). The reduction of proximal tubular Na^+ reabsorption during SVS infusion decreases tubular HCO_3 reabsorption, resulting peritubular cappilaries might become acidosis. This might result the preferential exchange of Na^+ with H^+ rather than K^+ at distal nehpron (Vander, 1995). Taken as a whole, the depression of proximal tubular K^+ reabsorption coincided with the reduction of distal tubular K^+ secretion results slightly or no change of K^+ excretion. However, urinary acidification should be determined to support this possibility.

SVS intubation also induced urinary electrolyte excretion without changes of renal hemodynamics reflecting its direct tubular effect. The general appearance of renal tissue observed from histopathological examination was quite normal which was different from SVS infusion. Proximal tubular defect is not possible because there was no glucouria along with natriuresis and diuresis (see in chapter VI). SVS feeding therefore should exert the effect at the other part of nephron. The direct influence or hormonal changes that elicit the change of tubular reabsorptive function at distal

nephron like arginine vasopressin (AVP), aldosterone and antinatriuretic peptide (ANP) are all possible. However, the inhibition on aldosterone effect is unlikely because K^+ excretion was enhanced instead of reduced. It was shown by the present experiment that SVS produced diuresis and elevation of electrolyte ,resulting the significant rise of plasma electrolytes concentration, Cosm and plasma osmolality. Therefore, it was presumably that SVS feeding reduces electrolyte reabsorption at distal nephron, resulting osmotic force to induce diuresis.

In conclusion, SVS both infusion and feeding produced hypotension, diuresis and natriuresis. Hypotensive response to SVS infusion was likely due to vasodilatation not the reduction of plasma volume. The elevation of renal blood flow without change of GFR during SVS infusion indicates the vasodilation of both afferent and efferent arterioles. Glomerular capillaries congestion was apparent in rats subjected to SVS infusion whereas slightly change in SVS intubation group. This might be the possible cause of the reduction of GFR after the end of SVS infusion. No signicant change of renal hemodynamics in rats fed with SVS. The natriuresis induced by SVS infusion should be its direct tubular action, since there were no change of GFR and plasma Na^+ concentration during SVS infusion. The reduction of proximal tubular Na^+ reabsorption should be the possible cause since there was the elevation of urinary glucose excretion in both during and after SVS infusion. In contrast, the increase of electrolyte excretion after SVS feeding was not likely due to the reduction of proximal tubular Na^+ reabsorption since there was no elevation of urinary glucose excretion. The mechanism responsible to natriuresis has not yet elucidated by the present experiment.