CHAPTER VI

THE EFFECT OF STEVIOSIDE ON GLUCOSE METABOLISM AND RENAL GLUCOSE HANDLING

Introduction

Glucose is the predominant nutrient for cellular energy supply. Several lines of evidences demonstrated the variation of plasma glucose level in response to SVS administration (Kinghorn and Soejarto, 1985). So far, no experimental evidences have supported the effect of SVS infusion on plasma glucose level and glucose kinetics. From the previous experimental results in chapterIV, SVS infusion produced hypotension and natriuresis. However, It is not known whether the glycemic effect of SVS infusion is associated with its renal and hypotensive effects. Furthermore, it has not yet been determined whether its various actions are direct effects or via mediators. It has been demonstrated that hypotensive and renal effects of SVS were partly dependent on prostaglandin (Melis and Sainati,1991a). Several agents including prostaglandin, nitric oxide, arginine vasopressin and angiotensin II have been shown to affect the plasma glucose level including blood pressure and renal functions (Robertson,1988; Chen et al., 1994; Jun and Wennmaln,1994;Manning et al, 1994).

Little data are available to demonstrate the effect of SVS on renal tubular glucose transport although intravenous infusion of SVS has been shown to enhance glucose clearance (Melis, 1992a). Normally, renal glucose reabsorption is Na^+

dependent, and SVS has been shown to produce natriuresis (chapter IV). Nevertheless, how and what mechanism SVS administration affects on renal glucose transport. The objective of this experiment was therefore to clarify the mechanism of SVS infusion on the blood glucose level and renal glucose handling. In addition, it sought to clarify whether the glycemic action of SVS is mediated by several agents which was related to renal function and blood pressure. The change of glucose kinetics during SVS infusion was also examined.

Materials and methods

Male Wistar rats weighing between 250-300 g were used. Normal rat food and tap water were supplied ad libitum. The general animal preparations were shown in chapter III. The experiments were divided into 4 series.

Series I Effect of SVS on plasma glucose level and renal glucose handling.

The animals were divided into 3 groups according to route and dose of SVS administration. Eight animals in each group were used.

Control group After surgical preparation as explained in chapter III, the animals received normal saline (NSS) intravenously throughout experimental period with the rate of 1 ml/100 g.BW. Five 30-min periods of experiment were carried out. Arterial blood samples (0.3 ml) and urine samples were collected to determine plasma glucose concentration and urinary glucose excretion in each period.

SVS infusion group The animals were divided in to 3 subgroups according to the dose of SVS infusion. The general procedures were similar to control group.

Five 30-min periods were undertaken for one control, first and second SVS infusion, first and second recovery period. After the first control period, SVS solution (150 mg/ml in NSS) was infused in 3 subgroups animals with a priming dose 100 , 150 and 200 mg/kg.BW, and followed by the rate of 100, 150, 200 mg/kg.BW/hr respectively for 1 hour.

SVS intubation group In this group, rats were divided into 2 subgroups including control and SVS feeding group. Feeding maneouver was separated into 2 consecutive 45-min periods. Following 6 hours of distilled water (control) or 2 g/kg.BW of SVS (150 mg/ml) was fed to animals, the general animal preparation was performed. This time interval was chosen since plasma level of SVS was highest at 6-8 hours after SVS ingestion, and this dose produced hypotension and natriuresis as shown by our previous experiment (chapter IV).

Series II Determination of the glucose turnover rate (GTR)

This series of experiments was carried out to assess the mechanism of action of SVS infusion on glucose kinetics by determination of glucose turnover rate (GTR). The principle of GTR has been accepted and used since 1959 (Steele, 1959 ; Katz et al., 1974 ; Umpleby and Sönksen, 1987). Sixteen animals were divided into 2 groups including control and SVS infusion group. According to the experiment in series I, the results of SVS infusion at the rate of 200 mg/kg.BW/hr showed a high response for an increase in plasma glucose level. Therefore the rate of infusion, 200 mg/kg.BW/hr of SVS was chosen for the treatment in the series II of experiments. Either SVS or NSS (control group) was intravenously injected throughout the experiment. After SVS (200

mg/kg.BW) or NSS was intravenously introduced for 30 min in each group, 0.2 ml of the tracer solution containing 1 μCi (U-^{14}C)-glucose and 5μCi (3-^{3}H)-glucose was infused as a priming dose and followed by continuous infusion of the tracer solution containing of 1 μCi/ml of (U-^{14}C)-glucose and 5 μCi/ml of (3-^{3}H)-glucose with the constant rate of 40 μL/min. The continuous infusion of the tracer solution was undertaken for a period of 90 min. This procedure produced a constant of radiospecific activity of glucose in the plasma about 60 min after the infusion of isotope-labelled glucose. An arterial blood (0.5 ml) was withdrawn every 15 min at 30-90 min of isotope-labelled glucose infusion to analyze GTR, percent glucose carbon recycling, glucose clearance and plasma glucose concentration as previously calculated in chapter III. After 30 min of SVS or NSS infusion, plasma insulin and glucose concentration were measured.

Series III The effect of preatment with drugs (including angiotensin II, arginine vasopressin, N ω-nitro-L-arginine methyl ester, indomethacin, prazosin and insulin) on the plasma glucose level during SVS infusion.

This series of experiments was carried out to elucidate whether the glycemic action of SVS is a direct effect or mediated via other mediators related to the hypotensive, diuretic and natriuretic action of SVS. The animals were divided into 8 groups, and eight animals in each group were used. After surgical preparation, the animals were allowed to eqilibrate for 45 min before the beginning of experiment. Four 30-min periods were estrablished including control period, pretreatment period and two periods of the combination infusion of SVS and various agents. No recovery

period was studied in this experiment because the glycemic response to SVS infusion was stable since the second period of SVS infusion (table 6.1)

Group I Animals recieved SVS infusion alone.

After NSS was intravenously infused for 1 hour, then 150 mg/ml of SVS in NSS was infused with a priming dose of 200 mg/kg.BW, and followed by 200 mg//kg.BW/hr for a period of 1 hour. Plasma glucose level was determined at the mid point of each period.

Group II Animals pretreated with prazosin (∝-adrenergic blocker)

The general preparation was similar to group I. When NSS was infused for 30 min (control) then prazosin (100 μg/ml) was infused with the priming dose of 100 μg/kg.BW and continued by 200 μg/kg.BW/hr throughut the experiment. Following the first 30 min of prazosin administration, 200 mg/kg.BW. of SVS was infused and then continued with the rate of 200 mg/kg.BW/hr in associated with prazosin for 1 hour.

Group III Animals pretreated with angiotensin II (A_{II})

The protocols was similar to group II except that in the pretreatment period, the animals were infused with 0.3 μg/kg.BW of A_{II} (0.4 μg/ml) as a priming dose, and followed by 50 ng/kg.BW/min.

Group IV Animals pretreated with arginine vasopressin (AVP)

The same general procedures as for group II was undertaken except that 0.5 ng/kg.BW/min of AVP (28.57 ng/ml) was infused.

Group V Animals pretreated with N ω-nitro-L-arginine methyl ester (L-NAME)

The identical procedures were performed as group II excepted that L-NAME (1 mg/ml) was infused with a priming dose of 1 mg/kg.BW and continued by 50 μg/kg.BW /min.

Group VI Animals pretreated with indomethacin

The general protocols were the same as for group II except that indomethacin (5 mg/ml in 8% Na_2HCO_3) was infused with a priming dose of 5 mg/kg.BW and followed by 5 mg/kg.BW/hr.

Group VII Animals pretreated with the combination of L-NAME+ indomethacin

Procedures similar to those for group II were performed, except that the combination of L-NAME and indomethacin was infused with the same dose as in group V and VI.

Group VIII Animal pretreated with insulin

The same protocols as group II were manifested excepted that insulin was primed with the dose of 0.1 U/kg.BW and then followed by 10 mU/kg/min.

Series IV Determination of renal plasma threshold and tubular transport maximum of glucose(Tm_G).

This experiment was undertaken to clarify the mechanism of renal glucose handling during SVS infusion. After surgical preparation, 1% of inulin in NSS was intravenously infused throughout experiment with the rate of 1%kg.BW. Glucose loading was performed in every 15 min period by infusion of 10, 15, 17.5, 20, 22.5, 25, 27.5 and 30 gm% of glucose in NSS. This procedures raised plasma glucose level in stepwise manner to approximately 200-500 gm%. Arterial blood (0.2 ml) and urine sample were collected in every 10 and 15 min respectively after changing the concentration of glucose infusion. Renal plasma threshold of glucose and Tm_G were determined.

To compare Tm_G of normal rats and renal tubular reabsorption of glucose in rats treated with SVS infusion, SVS (150 mg/ml) was infused with a priming dose of 200 mg/kg.BW and continued by 200 mg/kg.BW/hr for 1 hr. Two further 30-min periods of recovery were also performed. Renal tubular reabsorption of glucose was determined

Statistical analyses

Values were presented as mean±SEM and percent changes. Statistical analysis was performed using either paired or unpaired t-test. Wilcoxon signed rank test also used for wide standard deviation of the mean. The correlation was determined by linear regression and correlation. Data were considered to be significant difference from control period or group at the level of $P<0.05$.

Results

The alteration of plasma glucose and urinary glucose excretion

Table 6.1 and figure 6.1 revealed the alteration of plasma glucose (P_G), urinary excretion of glucose (U_GV) and fractional excretion of glucose (FE_G). Plasma glucose level was quite constant throughout experiment in control group. SVS infusion raised P_G from 119.33±1.99 to 132.00± 4.37 mg% ($P<0.05$) from 118.60± 4.94 to 165.33±10.75 mg% ($P<0.01$) and 117.27±2.35 to 157.35±8.71 mg% ($P<0.01$) in rats infused with 100, 150, and 200 mg/kg.BW respectively. The P_G level was continuously increased during the second period of SVS infusion and recovery. The magnitude of P_G elevation was quite constant until the end of experiment in group received 200 and 150 mg/kg.BW./hr but rose to a lesser extent in the group treated at the rate of 100 mg/kg.BW./hr. However, P_G level in animal fed with SVS was not different from that of the control group (figure 6.1).

The similar pattern of an elevation of U_GV and FE_G was also brought about in response to SVS infusion whereas no changes appeared in the control group. In the first period of SVS infusion, FE_G was significantly raised from 0.11±0.02 to 0.16± 0.03 % ($P<0.05$) , from 0.08±0.01 to 0.17±0.03 % ($P<0.05$) and from 0.11±0.02 to 0.31±0.08% ($P<0.01$) in group infused with SVS 100, 150, and 200 mg/kg.BW. respectively. The elevation of FE_G was the dose-dependent manner. Similar features were taken place in the second period of SVS infusion. Although after the termination of SVS infusion, FE_G was remained high until the end of experiment in all

Table 6.1 Effect of SVS infusion on plasma glucose (P_G), urinary glucose excretion (U_GV) and fractional glucose excretion (FE_G).

Parameters \ Periods	control	SVS infusion		recovery	
	30 min	60 min	90 min	120 min	150 min
P_G (mg%)					
Control group	122.83±3.32	119.15±4.70	120.60±3.71	118.16±4.51	117.50±3.53
SVS infusion (100 mg/kg.BW)	119.33±1.99	132.00±4.37*	130.65±4.88	133.00±5.46*	133.81±6.75
SVS infusion (150 mg/kg.BW)	118.60±4.94	165.33±10.75**	159.49±6.39**	169.22±6.38***	165.82±6.04***
SVS infusion (200 mg/kg.BW)	117.27±2.35	157.35±8.71**	153.71±5.70***	164.06±3.60***	161.33±6.56***
FE_G (%)					
Control group	0.14±0.01	0.15±0.03	0.14±0.03	0.13±0.03	0.11±0.05
SVS infusion (100 mg/kg.BW)	0.11±0.02	0.16±0.03$^{\alpha}$	0.19±0.03$^{\alpha}$	0.21±0.03$^{\alpha}$	0.19±0.04
SVS infusion (150 mg/kg.BW)	0.08±0.01	0.17±0.03$^{\alpha}$	0.38±0.18$^{\alpha}$	0.35±0.13$^{\alpha}$	0.47±0.17
SVS infusion (200 mg/kg.BW)	0.11±0.02	0.31±0.08$^{\alpha\alpha}$	0.66±0.40$^{\alpha\alpha}$	1.19±0.59$^{\alpha\alpha}$	0.92±0.47
U_GV (μg/min/kg.BW)					
Control group	5.17±0.32	5.15±0.89	4.64±0.94	4.37±0.85	3.68±1.43
SVS infusion (100 mg/kg.BW)	6.38±1.07	11.46±1.61$^{\alpha}$	12.89±1.79$^{\alpha}$	13.67±2.32$^{\alpha}$	14.56±3.77
SVS infusion (150 mg/kg.BW)	6.85±1.67	14.61±3.54$^{\alpha}$	15.73±2.63$^{\alpha}$	18.76±4.15$^{\alpha}$	29.56±12.48
SVS infusion (200 mg/kg.BW)	6.93±0.98	21.35±3.60$^{\alpha\alpha}$	39.76±22.94$^{\alpha\alpha}$	73.27±42.22$^{\alpha\alpha}$	50.67±26.46$^{\alpha\alpha}$

The values are mean±SEM. Significant differences comparing to control period are shown by *P<0.05, **P<0.01, ***P<0.001. $^{\alpha}$ P<0.05, and $^{\alpha\alpha}$ P<0.01 are indicated by Wicoxon signed rank test . The number of rats in each group is 8.

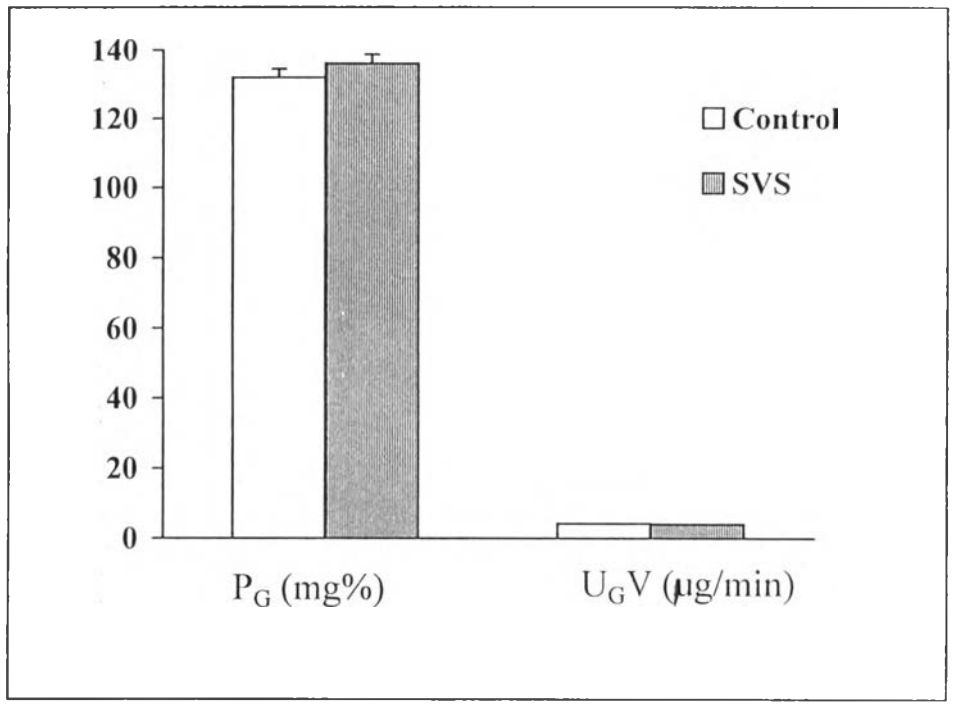

Figure 6.1 Alterations of plasma glucose(P_G) and urinary glucose excretion (U_GV) in rats given of SVS feeding. The number of rats is 8

groups. No significant alteration of both U_GV and FE_G occured after feeding with SVS (figure 6.1).

Effect of SVS infusion on glucose turnover rate (GTR)

The change in GTR, plasma glucose concentration and plasma insulin concentration are presented in table 6.2. The plasma glucose concentration was significantly increased from 120.32±5.93 mg% in control group to 176.8±10.8 mg% ($P<0.01$) in SVS infusion group whereas the plasma insulin level was not different between in the two groups. GTR of either (3-^{3}H)-glucose or (U-^{14}C)-glucose was slightly increased but not statistical significant difference from the control group. Glucose carbon recycling was significantly reduced from 28.7±1.3 to 23.0±1.6% ($P<0.05$). Glucose clearance fell from 6.46±0.34 ml/min/kg.BW in control group to 4.99±0.20 ml/min/kg.BW ($P<0.01$) in SVS infusion group which was reduced approximately 23 %. This extent was nearly the same as the rise of plasma glucose level (30%).

The effect of pretreatment with drugs on plasma glucose level during SVS infusion.

Table 6.3 and figure 6.2 demonstrate the effect of SVS infusion on the plasma glucose concentration (P_G) after pretreatment with angiotensin II (A_{II}), arginine vasopressin (AVP), L-NAME, indomethacin, prazosin and insulin. In control group which received SVS alone, P_G level increased from 117.19±2.89 to 151.37±1.81 mg% ($P<0.001$) during the first period of SVS infusion. The P_G level remained elevated to

Table 6.2 Glucose turnover rate (GTR) in rats subjected to normal saline (control) and SVS infusion (N=8). Significant differences comparing to control group are shown by *P<0.05, **P<0.01.

Parameters \ Group	control	SVS
Glucose turnover rate (μmol/min/kgBW)		
(3-^{3}H)-glucose	56.87±2.48	61.25±2.87
(U-^{14}C)-glucose	40.68±2.28	47.45±3.20
%Glucose carbon recycling	28.72±1.27	22.98±1.64*
Glucose clearance (ml/min/kg.BW)	6.46±0.34	4.99±0.20**
Plasma insulin ((μU/ml)	38.86±3.36	40.54±2.04
Plasma glucose (mg%)	120.32±5.93	176.80±10.77**

Table 6.3 Effect of SVS infusion on plasma glucose concentration (mg%) in rats treated with prazosin, angiotensin II, arginine vasopressin, L-NAME, indomethacin, L-NAME + indomethacin , and insulin. Significant difference from control period is indicated by *P<0.05, **P<0.01 and ***P<0.001.

Periods / group	control	drug or NSS	SVS + drug	
	30 min	60 min	90 min	120 min
Control	117.19±2.89	121.09±2.34	151.37±1.81***	153.83±2.49***
Prazosin	117.77±1.36	116.46±3.22	136.34±6.77*	137.83±8.59*
Angiotensin II	119.67±3.86	130.39±4.33***	151.90±8.19***	151.53±8.44***
Arginine vasopressin	120.26±2.94	125.21±5.16	152.23±4.87***	145.43±5.97***
L-NAME	118.29±2.57	117.68±3.50	152.94±5.47***	139.63±5.15**
Indomethacin	119.42±3.30	111.38±3.01*	148.47±3.34***	134.14±4.77*
L-NAME+Indomethacin	117.02±1.77	108.96±1.68***	132.83±6.26*	132.51±8.99
Insulin	119.46±2.15	99.44±4.12**	134.90±13.31	105.22±12.86

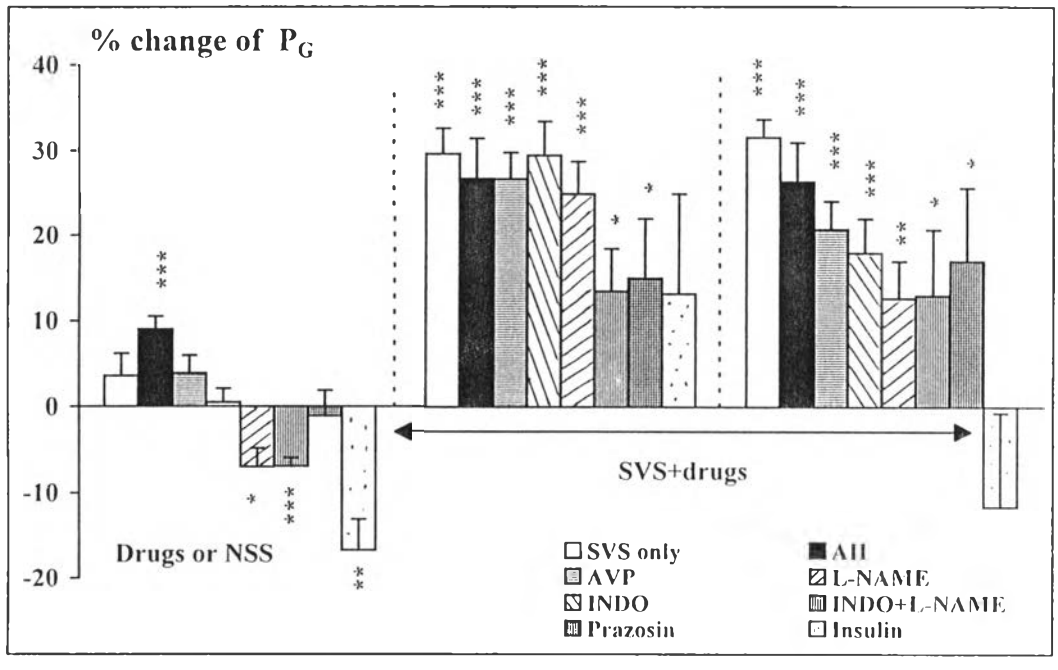

Figure 6.2 Percent change of plasma glucose level (P_G) in response to SVS infusion alone and pretreated with norepinephrine(NE), angiotensin II (A_{II}), arginine vasopressin (AVP), L-NAME, indomethacin (INDO), L-NAME+indomethacin (L-(NAME+INDO) and insulin. Significant differences are compared to control period are indicated by *P<0.05, **P<0.01, ***P<0.001. The numberof rats in each group

153.83±2.49 mg% (P<0.001) in the following period of SVS infusion. Animal pretreated with prazosin could have attenuated P_G levels, but still higher than that of control period (P<0.05) during SVS infusion. The rise of P_G level was remained appeared during SVS infusion(P<0.001) in rat pretreated with A_{II} and AVP. The hyperglycemic effect of SVS infusion was attenuated in the second period in rats pretreated with L-NAME. Pretreatment with indomethacin reduced P_G level from 119.42±3.30 to 111.38±3.01 mg%(P<0.05). The elevation of P_G level to 148.47±3.34 mg% (P<0.001) was apparent during the first period of animals treated with the combination of indomethacin and SVS infusion. Nevertheless, the elevation of P_G was attenuated to the level of 134.14±4.77 mg% (P<0.05) in the second period. Pretreatment with a combination of L-NAME and indomethacin gradually reduced P_G concentration from 117.02±1.77 to 108.96±1.68 mg% (P<0.001). The P_G level was slightly higher (P<0.05) during the first period but it was not significantly different from the control level during the second period of SVS infusion in animals treated with a combination infusion of L-NAME and indomethacin. Insulin administration reduced P_G level from 119.46± 2.15 to 99.94±4.12 mg% (P<0.01). No significant change of P_G level was observed during either the first or second period of combination infusion of SVS insulin.

Determination of renal plasma threshold and renal transport maximum of glucose (Tm_G)

The renal capacity for glucose reabsorption was determined by loading glucose into blood circulation. The average of Tm_G was 9.07±0.42 mg/min.(table 6.4)

whereas the tubular reabsorption of glucose in rat subjected toSVS infusion was approximately 1.5-2 mg/min (table 6.5). During recovery period, renal tubular reabsorption of glucose in rats treated with SVS infusion tended to reduce but not significant difference from control. Figure 6.3 illustrates the correlation between plasma glucose concentration and urinary glucose excretion. Renal plasma threshold of glucose was found to be approximately 264±19.25 mg%.

Discussion

In the present study, SVS infusion produced a rise in the plasma glucose level (P_G). The hyperglycemic response to SVS infusion was maintained until the recovery period. The time effect did not account for this change because there was no alteration of the P_G level throughout the experiment in control animals. An increase in exogenous glucose during SVS infusion would also not explain it since there is only a small amount of glucose (3 mg/100 mg SVS) contained in SVS infusate. Furthermore, the P_G level was remained high despite the lack of SVS administration during the recovery period. This indicates that SVS's effect remained despite stopping the SVS infusion into the body. This suggestion is supported by the result of Ishü and Brach (1995) that the concentration of SVS remained constant after 2 hours of SVS infusion into isolated rat liver. Therefore, the hyperglycemic appearance during and after SVS infusion should be the effect of SVS per se. In the present experiment, no significant change in P_G was observed in rats subjected to SVS intubation. Similar results were found by Lee and his co-workers (1979). However, the controversial results were also noted for a significant decrease in blood glucose level in humans given an aqueous

Table 6.4 Tubular transport maximum of glucose (T_{mG}) in normal rat loaded with intravenous infusion of various concentration of glucose.

rat No	T_{mG} (mg/min)
1	11.03
2	9.69
3	8.67
4	12.29
5	10.39
6	10.78
7	9.91
8	8.83
mean ± SEM	9.07±0.42

Table 6.5 The alteration of tubular reabsorption of glucose (mg/min) in rat before, during and after 200 mg/kg.BW of SVS infusion (N=8).

control	SVS infusion		recovery	
-30 min	30 min	60 min	90 min	120 min
1.84±0.21	2.22±0.32	1.89±0.24	1.63±0.27	1.55±0.31

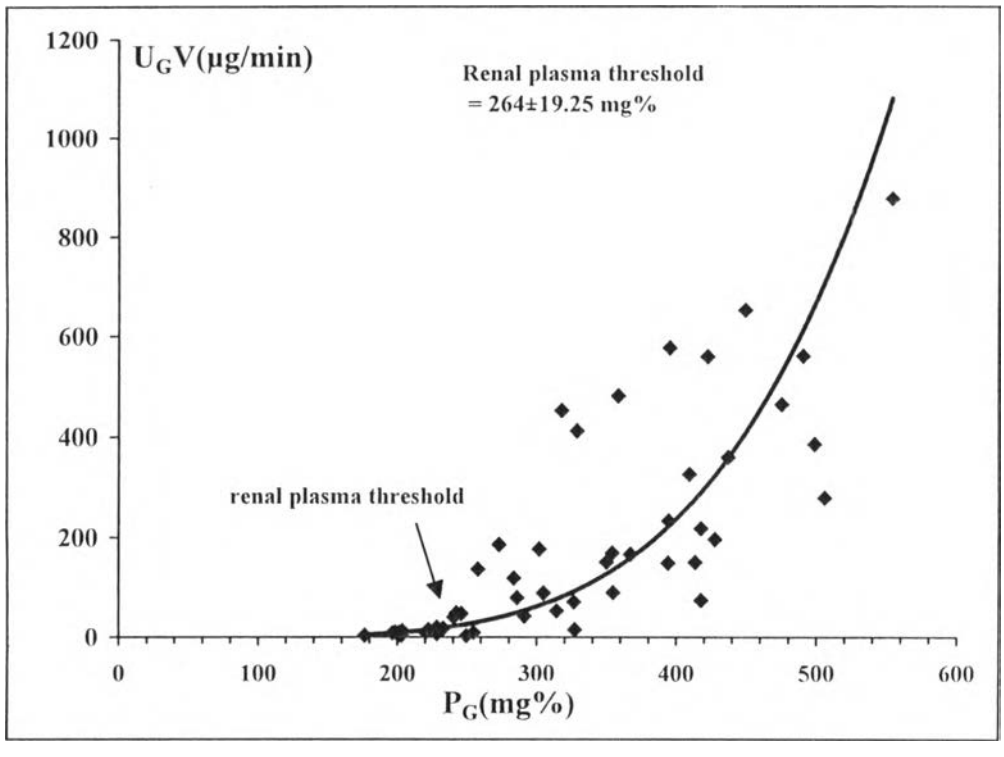

Figure 6.3 The relationship between plasma glucose (P_G) and urinary glucose excretion (UGV) after glucose loading. The number of rats is 8.

extract of Stevia orally (Curi et al.,1986), and hamster fed with SVS for 12 hours (Toskulkao and Sutheerawattananon, 1994). The inconsistent results reported by various studies probably depend on the dose employed, time course, conditions, and experimental model. The unalteration of the plasma glucose level in rats fed with SVS in the present experiment was probably due to the conversion of SVS feeding to steviol in rats (Nakayama et al., 1986). Although steviol has been reported to inhibit glucose absorption in isolated jejunum, its effect has been shown to be related to the duration and concentration of steviol administration (Toskulkao et al., 1995a). In addition, the dose and duration of SVS intubation in this experiment might not have been high enough to affact the plasma glucose level (Toskulkao and Sutheerawattananon, 1994). The dose and duration of SVS feeding used in the present experiment were chosen because of the hypotensive and natriuretic response as demonstrated from our previous experiment (chapter IV).

The mechanisms responsible for the hyperglycemic action of SVS infusion have not yet been elucidated. Several mechanisms have been proposed. The facilitation of intestinal glucose absorption induced by SVS infusion should not a possible cause, since SVS had no effect on glucose absorption in hamster jejunum (Toskulkao et al., 1995b). In the present experiment, SVS infusion had no effect on the plasma insulin level despite hyperglycemia (table 6.2). However, the administration of insulin was able to inhibit the hyperglycemic effect of SVS infusion. These results indicate that tissue response to insulin during SVS infusion is still intact, and the elevation of the P_G level is not due to the reduction of insulin liberation. It is quite clear that the appearance of hyperglycemia should be the result of SVS itself. In

the present results, insulin release was not increased despite the elevation of the P_G level, although it has been found that the rise of the P_G level above 80 mg% would stimulate insulin synthesis and release (Bullock, 1995d). This implies that SVS abolishes insulin response to hyperglycemia. Usami and his co-workers (1980) also found that SVS did not directly affect the arginine-stimulated insulin or glucagon release from isolated pancreases. The possible mechanism that SVS inhibited insulin response to hyperglycemia is explained. Insulin release in response to hyperglycemia has been reported to be dependent on the rise of intracelluar Ca^{2+} ($[Ca^{2+}]_i$) in pancreatic β-cells (Black et al.,1994). The Ca^{2+} channel antagonist decreased glucose-induced insulin secretion along with the prevention of $[Ca^{2+}]i$ rise (Black et al.,1994). SVS infusion has been demonstrated to act as a Ca^{2+} channel blocker to induce hypotension and natriuresis which were reversed by Ca^{2+} supplements (Melis and Sainati,1991b). Thus, it is quite possible that SVS infusion blocks Ca^{2+} uptake to pancreatic β-cells, resulting no response to hyperglycemia.

The glucose turnover rate (GTR) did not significantly change but there was a reduction of glucose clearance along with the elevation of the P_G level. These reflect the underutilization of glucose during SVS infusion. The inhibition of glucose transport across the cell membrane by SVS in the perfused rat liver has also been noted (Ishü et al., 1987). This supports the direct inhibitory influence of SVS infusion on glucose uptake which finally provides hyperglycemia. Nevertheless, various hormonal changes in response to SVS infusion which may influence the P_G level should not be excluded.

SVS infusion has been shown to reduce blood pressure (Melis and Sainati,1991a,b). The hyperglycemic response to SVS infusion may be the result of sympathetic compensation to its hypotensive effect. Bullock (1995c) reported the inhibitory action of norepinephrine (NE) on glucose-induced insulin secretion from the islets of Langerhans. Norepinephrine also inhibited insulin-mediated glucose uptake in the perfused hindlimb of the rat (Rattigan et al., 1995). Thus, the sympathetic adrenergic nerve plays a significant role in raising the P_G level and inhibiting glucose-induced insulin secretion which was likely similar to the action of SVS infusion. From the present result, prazosin, a α-adrenergic antagonist, attenuated hyperglycemic response to SVS infusion. This indicates that sympathetic stimulation does at least in part participate in hyperglycemic action and inhibition of glucose-stimulated insulin release induced by SVS infusion.

It has been shown that nitric oxide (NO) and prostaglandin E produced hypotension and natriuresis (Manning et al.,1994; Baer, 1988) which was likely similar to the effect of SVS infusion (Melis and Sainati,1991a). L-arginine ,the precursor of NO synthesis, could elevate the P_G level, which was reduced by pretreatment with L-NAME ,a NO synthesis inhibitor (Jun and Wennmaln, 1994). In addition, NO was demonstrated to abolish glucose-stimulated insulin release from isolated rat pancreatic islets by the closure of ATP-sensitive K^+ channel which is necessary for Ca^{2+} uptake to β-cell (Tsuura et al., 1994). Thus, it may be possible that the hyperglycemic action and inhibition of glucose-induced insulin release by SVS infusion would be mediated via NO synthesis. However, there is no experimental evidence to support the mechanism of hyperglycemic action of NO. The present result

showed that L-NAME reduced the hyperglycemic action of SVS infusion in the second period. This indicates that NO partly participated in the hyperglycemic action of SVS infusion. However, the mechanisms of its action cannot be clearly explained by the present experiment. The possibility that L-NAME pretreatment increases insulin secretion would be unlikely since there was a reduction in the insulin level along with the decrease of the plasma glucose level during L-arginine infusion after pretreatment with L-NAME. (Jun and Wennmaln, 1994). It is unknown why L-NAME pretreatment could not completely reverse the hyperglycemic effect of SVS infusion. The same result was also demonstrated in rats subjeted to L-arginine after pretreatment with L-NAME (Jun and Wennmaln, 1994). The delay of the effect of L-NAME on the hyperglycemic action of SVS in the present result might be due to the delay of cGMP production induced by NO (Laychock et al., 1991). Although L-NAME pretreatment reduced the hyperglycemic action of SVS, the plasma glucose level did not return to a normal level. This indicates that there are some other mechanisms responsible for the hyperglycemic effect of SVS.

Prostaglandin has been shown to suppress insulin release, raise glucagon and P_G levels, and reduce glucose-stimulated insulin release (Robertson, 1988; Band et al., 1993). From the present experiment, indomethacin reduced the P_G level during pretreatment and the second period of SVS infusion. This indicates that prostaglandin would be associated with an increase in P_G level during SVS infusion. However, from the result of the GTR study, the elevation of the P_G level during SVS infusion should be primarily due to the reduction of glucose uptake rather than the decrease of the insulin level. Therefore, a direct inhibitory effect of prostaglandin on glucose uptake

rather than the reduction of insulin release should probably be the major cause of hyperglycemia during SVS infusion. To support this possibility, aspirin, a prostaglandin synthesis inhibitor, has been reported to enhance peripheral glucose uptake (Vallecorsi et al., 1964). Moreover, inhibition of prostaglandin systhesis has been reported to increase glucose-induced insulin secretion (Metz et al., 1981). Thus it seems that the reduction of hyperglycemia during SVS infusion in rat pretreated with indomethacin might be due to the secretion of insulin. To clarify this possibility, the glucose turnover rate and insulin level should be further investigated in animals treated with the combination infusion of indomethacin and SVS.

Although indomethacin pretreatment alleviated the elevation of the P_G level during SVS infusion, it was still higher than that of the control, and took more than 30 min. The same response on renal function was reported by Melis and Sainati (1991a). The inability of indomethacin to reverse the hyperglycemic effect of SVS is not due to inefficiency in inhibiting prostaglandin synthesis, since around 90% of prostaglandin synthesis was abolished by this dose of indomethacin within 30 min after administration (Kramp et al.,1995). The present experiment showed that sympathetic activity might participate on the hyperglycemic effect of SVS infusion. It has been reported that indomethacin partially reversed the α-adrenergic inhibition on insulin response to high glucose (Metz and Robertson, 1980). This may be the reason why indomethacin pretreatment could not completely reverse the changes of P_G during SVS infusion.

It is interesting that animals pretreated with the combination of L-NAME and indomethacin had a P_G level that decreased more than after the pretreatment of

indomethacin or L-NAME alone. It seems that L-NAME potentiates the effect of indomethacin to reduce P_G level. Supporting this interpretation is the further reduction of the P_G level both before and during SVS infusion compared to animals pretreated by indomethacin or L-NAME alone. The mechanisms underlying this response are not known.

In conclusion, the effect of SVS infusion on plasma glucose levels is not due to the reduction of the plasma insulin level. It should be due to the effect of SVS on glucose transport across the cell. SVS can inhibit glucose-stimulated insulin release. NE, prostaglandin and NO were found to participate in the hyperglycemia and the inhibition of glucose-stimulated insulin release action of SVS infusion. However, the explanation that these drugs are responsible for these actions is not supported by the present experiment, since the hyperglycemic effect of SVS infusion was not completely reversed in animals pretreated only with α-adrenergic blocker, indomethacin or L-NAME. Therefore, there might have some interactions among NE, NO and prostaglandin on the hyperglycemic action of SVS infusion.

SVS infusion caused not only natriuresis but also glucosuria. Similar results was achived by Melis (1992a). It has been reported that subcutanous injection of SVS elevated urinary glucose excretion in associated with the degeneration of proximal tubule (Toskulkao et al, 1994). The urinary glucose excretion may be the result from a wide variety factors, for example, the rise of P_G level, the impairment of proximal tubular Na^+ reabsorption, the integrity of reabsorptive cell, etc. The elevation of P_G level during SVS infusion was not as high as renal plasma threshold (figure 6.3),

hence, it is not to be the cause of glucosuria. Tubular reabsorption of glucose during SVS infusion was markedly lower than Tm_G (table 6.4, 6.5). Thus, glucosuric response to SVS infusion is not due to the maximum tubular reabsorption of glucose. Other possible mechanisms were postulated. In general, renal tubular glucose reabsorption should be proportional to the P_G until renal plasma threshold. Renal tubular glucose reabsorption was quite constant during SVS infusion and slight reduced during recovery period. These indicate that the glucosuric appearance is the result from the reduction of renal tubular glucose reabsorption. Normally, renal glucose reabsorption is Na^+-dependent process (Deetjen et al., 1992 ; Ganong, 1995). Furthermore, renal glucose reabsorption is nearly completely reabsorbed by Na^+-glucose co-transport at proximal tubule. The distal nephron have a certain capability for glucose reabsorption but their quantitative contribution to total nephronal transport is almost negligible (Deetjen et al., 1992). The rise of FE_G was closely correlated to natriuresis (previously shown in chapter IV and table 6.1), supporting Na^+-dependent glucose reabsorption in response to SVS infusion. The profound glucosuric appearance during and after SVS administration also confirms the proximal tubular natriuretic influence of SVS infusion (chapter V).