CHAPTER II

BACKGROUND INFORMATION

Stevioside (SVS)

SVS and extracts prepared from the leaves of the plant Stevia rebaudiana or Stevia have been widely used in Japan since mid 1970s as sweetening agents for sugar substitute. This plant is well grown in the area of elevated terrain at lattitude ~25°S in the Amambay and Iguacu districs on the border of Brazil and Paraguay (Kinghorn and Soejarto, 1985). The distribution of this plant extends from south-western United States to Northern Argentina, through Mexico, Central America, the South American Andes, and the Brazil highlands. In 1900, Rebaudi reported the general chemical structure of sweetenner from Stevia that was glycoside. Although Stevia composes of several sweet glycoside, such as rebaudioside A-E, dulcoside A, steviobioside, SVS has the great sweetness approximately 100-300 times sweeter than sucrose (Kinghorn and Soejarto, 1985 ; Hanson and Oliveira, 1993). Also, SVS is the major constituent extracted from Stevia. It accounts for 6-10% in dried leaves.

The native of Paraguay and Brazil have been using the leaves of Stevia as a sweetening agent and contraceptive drug for centuries. SVS is now cultivated commercially in Brazil, Israel, Korea, China and Japan (Hanson and Oliveira, 1993). Japan was the first country in Asia that brought it to be consumed, and now for commercial uses. In 1987, it was estimated that about 1700 tonnes of leaves were

used in Japanese food products. Extracts, sold under various name such as Stevix, Steviosin are used in drinks and other food products (Fujita, et al, 1977 ; Hanson and Oliveira, 1993). According to its well grown in high light intensities, with warm temperatures, minimal frost and water ; some area in Thailand is the suitable area for cultivation. Now a day, the Northern part of Thailand including Chieng Rai, Chieng Mai, Phayao and Lumpoon provinces have cultivated Stevia, and helps the farmer to receive fairly high income (~7,000-9,000 Bath per rhai). However, the cultivated Stevia in Thailand has not been used to consume within the country but only sent to be crude product to Japan. It may be used in the near future in Thailand if more information about the safety uses is available.

1. Chemical and Physical properties

SVS is a light, colorless and odorless powder with molecular weight of 804.9 and has empirical formula of $C_{38}H_{60}O_{18}$ (Fig.2.1) or 19-O-β-glucopyranosyl-13-O-[β-glucopyranosyl-(1-2)]-β glucopyranosyl-steviol or steviol-13-O-β-saphoroside-19-O-β- D-glucopyranosyl ester (Glinsukon et al., 1988a). SVS consists of β-glucosyl and β-sophorosyl group bound to C_4 and C_{13} positions of a diterpenic carboxylic alcohol called aglycone, steviol or cyclopentanoperhydrophenanthrene. After enzymatic hydrolysis, SVS gives 1 mole of steviol and 3 moles of D-glucose (Wingard et al., 1980). SVS is highly stable over the pH range 3-9 or heated at 100°C for 1 hour but rapid decomposed under pH level greater than 9 (Kinghorn and Soejarto, 1985). It's melting point is approximately 198°C. SVS does not change to dark or brown color either by aqueous solution or solution with amino acid being heated. It slightly soluble in ethyl and methyl alcohol, and less soluble in H_2O but quiet soluble in

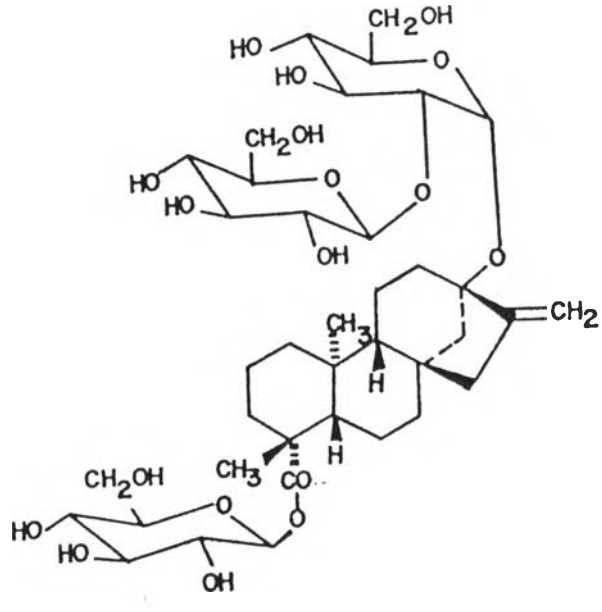

Fig. 2.1 The chemical structure of stevioside. (Glinsukon et al, 1988)

dimethyl sulfoxide (DMSO). It is almost insoluble in propylene glycol, ethyl acetate and chloroform (Glinsukon et al., 1988a). SVS seems to be relatively stable in food substances at slightly elevated temperature, but quiet decomposed at higher temperature of storage (Kinghorn and Soejarto, 1985).

2. Absorption, distribution, metabolism and excretion

SVS likes oligosaccharide that it is a non-absorbable sugar. It was demonstrated that SVS was degraded by rat caecal microflora to steviol *in vitro* (Glinsukon et al, 1988a). Nakayama et al (1986) detected glucose and steviol in rat caecal content at 24 hrs after oral admistration of ^{3}H-SVS. The level of ^{3}H-SVS in blood was measured to maximum level at 8 hours after its administration. The radioactivity was found to be totally degraded in ceacal content at 24 hours after administration but it still appeared in blood at 48 hours. Steviol was likely to be the major metabolite which was derived from degradation of SVS in intestine and biliary excretion (Wingard et al, 1980). The radioactivity was mainly excreted into feces and expired air and only a small amount in urine. The degradation of SVS to steviol may be predicted to occur in human because the flora of rat intestine and lower human bowel are quantitatively and qualitatively similar (Luckey, 1972), and steviol would be completely absorbed via the gastrointestinal tract. However, it is not yet known whether steviol by itself exerts toxicological and biological actions.

3. Safety and Toxicity of SVS and Stevia extract

3.1 Acute toxicity

In early work, SVS as purified by Bridel and Lavielle in 1931 was reported to

be nontoxic to the rabbit, guinea pig, and fowl. The LD_{50} of an extract of Stevia leaves containing 50% SVS was indicated to be 3.4 g/kg.BW when administered intraperitoneal (ip) to rats (Lee et al., 1979). On the other hand, the later researches demonstrated that its toxicological effects depends on dose administration, purity, route of delivery and animal species. Lee and co-workers (1979) reported that SVS has an LD_{50} of 8.2 g/kg.BW when administered orally to rats. LD_{50} for hamster and guinea pigs are 4.1-6.15 and >4.1 g/kg.BW respectively when given orally (Glinsukon et al., 1988b ; Fujita and Edahiro, 1979). Kurahashi et al. (1982) demonstrated that LD_{50} of SVS (69% purity) in Stevia extract is highest in mice (0.89 g/kg.BW) when given intravenously. For most studies, LD_{50} are in range 1-3 g/kg.BW when ip administration (Glinsukon et al., 1988a), and rat seems to be more susceptable to SVS than mice whereas hamster is most sensitive to SVS when given ip (Glinsukon et al, 1988a,b). However, no acute toxicity appears in mice when 2 g/kg.BW of SVS was administered orally. From these experiment, it seems to be that SVS is least toxic when given by oral route to rat and mice except hamster but it is quiet toxic when give iv and ip route.

The general sign and symptons expressed when given with lethal dose have been reported by several studies. In hamster, SVS (4.1 g/kg.BW orally) caused the lower urinary excretion in along with the significant increase of plasma level of blood urea nitrogen (BUN), creatinine, uric acid, PGPT and PGOT after 6 hours of SVS administration (Panichkul et al., 1988). The damaging in the area of proximal tubule was also reported. It is suggested that the possible causes of death induced by SVS in hamster and guinea pigs is likely to be due to acute renal failure (Glinsukon et al.,

1988a). In mice and rat, histopathological changes are commonly observed. These include of congestion, degeneration and impairment to the vital organs such as heart, lung, liver, brain and kidney (Kurahashi et al., 1982 ; Glinsukon et al., 1988a ; Deechawan, 1992). The possible causes of death may be associated with respiratory failure and cardiac dysfunction. No direct relation between nephrotoxic effects and lethal causes of death in mice and rat (Glinsukon et al., 1988a).

3.2 Subacute toxicity

Mitsuashi and his co-workers (1976) performed study by oral aministration of SVS in dose of 0.1, 0.5 and 2.5 g/kg.BW for a period of one month in female rats. They didn't find any organ abnormalities. Similarly, Akashi and Yokoyama (1975) reported that neither deaths nor any particular symptoms was noted in both male and female rats treated with SVS in food up to 7% throughout 3 months. A similar study was carried out in South Korea with an aqueous extract of Stevia containing 50% SVS. Each rat recieved 0.2-0.5 g SVS daily for 56 days. No abnormalities are reported either blood chemistry or histopathological examination (Lee et al., 1979).

3.3 Chronic toxicity

Yamada et al. (1985) demonstrated that no significant alterations were elicited in the growth, organ weight, blood chemistry and histopathological examination in both male and female rats subjected to SVS orally (92.5% purity) at the dose of 550 mg/kg.BW/day for 22-24 months. Similar negative results were also obtained in both male and female Syrain Golden hamster given SVS 1 g/kg.BW for 6 month and continuously feed with normal pellets for additional 6 months (Glinsukon et al.,

1988a). Furthermore, SVS is not carcinogenic to rats and hamsters. Xili et al (1992) recently studied the effect of SVS (85% purity) given in diet at 0.2-1.2% for 2 years. No significant changes of growth, food consumption, general appearance, mortality rate, hematological, urinary or clinical biochemical values at any stage of study.

For mutagenic influence of SVS has interested so many researchers. Most of the mutagenic testings in several animal species treated with SVS or crude extracts showed the negative results. For instance, Pimbua et al. (1988) found that SVS (20 mg/plate) could not induce mutagenicity toward S-typhimurium TA98 and TA100 with metabolic activation of mouse, rat, hamster, guinea pig and rabbit liver S-9 fractions. The same results was achived by Tama Biochemical Co., Ltd. using crude extracts or SVS. However, its metabolite, steviol, was found to have mutagenic and bactericidal activity to S. typhimurium TM677 when evaluated in the presence of S-9 fraction from the liver of Arochlor 1254-pretreated all above animals (Pezzuto et al., 1985).

4. Biological and pharmacological effect of SVS

4.1 Effect of SVS on reproduction

It has been reported that the native of Paraguay and Brazil used Stevia leaves and stems as an oral contraceptive for centuries. Plana and Kue (1968) reported the antifertility effect of aqueous extract of Stevia following the addition of 5% extract in 10 ml of water for a period of 2 months in both male and female rats. However, the latter studies have shown the lack of antifertility effect. SVS (95-98%) was given in diet at a concentration of 0.15-3% before mating and during the early period of

gestation had no any effect either on fetility or development of fetus (Mori et al., 1981). The same results were again observed in hamster treated with SVS 1-2.5 g/kg.BW/day (Yodyingyuard and Bunyawong,1991). Thus, it is likely that SVS has no any fertility influence in both rat and hamster.

4.2 Effect of SVS on neural and muscular system

Kurahashi and his colleagues (1982) found that no difference of sleeping time was obtained when 70 mg/kg.BW of hexobarbital was injected ip into male mice following oral consumption of 0.5 g/kg.BW of SVS (93% purity). Likewise, there was no statistical significant difference of writhing syndrome when 0.1 ml/100 g.BW of 0.6% acetic acid was injected ip into male mice taken 2 g/kg.BW of SVS (69% purity). Furthermore, no changes were detected either 0.2 ml of 5% SVS was dropped in an eye of male rabbit or 20% application to the skin of male mice. Notwithstanding, SVS at the concentration of 10^{-4}M (83% purity) has been reported to greatly enhance the contractile response of guinea pig ilium in vitro stimulated with K^+ (165 mM), Ca^{2+} (0.3 mM) or Ach (Kinghorn and Soejarto, 1985). In contrary, SVS at the concentration of 4 mg/ml (69% purity) inhibited rabbit and guinea pig intestinal contraction when stimulated by histamine in vitro.

4.3. Effect of SVS on carbohydrate and energy metabolism

It has been claimed that the extracts of Stevia plants was used to be popular medicine in Paraguay (in form of tea) as a remedy for diabetes, indicating the hypoglycemic effect of Stevia. However, all datas supported this influence have been published only in the form of abstracts, with little evidence. Moreover, the

hypoglycemic action is almost shown in subjects with treatment of Stevia extract whereas so little information known for SVS's effect.

The earliest study of hypoglycemic effect of SVS was manifested by Oviedo and co-workers (1970). They showed an average 35.2% fall of blood glucose level in 25 healthy adult given aqueous extract of Stevia without specifying dose for a period of 6-8 hours. Further study was performed by Suzuki and his collegues (1977). They found a significant reduction of liver glycogen and blood glucose (119±3.1 to 100±8 mg%) in rats fed with a high carbohydrate diet containing 10%dried Stevia leaves (corresponding 0.5% SVS in diet) for 4 weeks. Moreover, 0.1% SVS that added to high carbohydrate diet resulted a drop of liver glycogen without change of blood glucose whereas no significant change of both parameters in rat fed with high fat diet. Curi et al. (1986) reported that aqueous extract of Stevia raised glucose tolerance test in normal volunteers subjected to oral administration of 5 g aqueous extracts at regular 6 hours for 3 days. On the other hand, the controversial outcomes are also reported. Lee and co-workers (1979) reported that blood glucose was quiet normal when crude extracts of Stevia were given to rats for 56 days, each rat consumed 0.5-1 g/day. Similarly, no dose-related effect on blood glucose was apparent following feeding the rat with 7% Stevia extract for 56 days. The same results was achived in alloxanized rat. Nonhypoglycemic effect of SVS was again supported by the experiment that 15 μM of SVS did not directly influence on the arginine-stimulated insulin or glucagon release from isolated pancrease (Usami et al.,1980). From several experimental results mention earlier, there are some conflicing results about glycemic effect of Stevia extract and SVS.

Vignais et al. (1986) sowed the effects of SVS and other component of Stevia on oxidative phosphorylation in isolated mitochondria. Steviol and dihydrosteviol exhibited an inhibitory effect on oxidative phosphorylation without any uncoupling effect. Similarly, Bracht et al. (1985a,b) also reported the inhibitory effect of SVS on oxidative phosphorylation, ATPase, NADH-oxidase activity, succinate-oxidase activity, succinate dehydrogenase, L-glutamate dehydrogenase, and lowered of ADP/O ratio in isolated rat liver mitochondria. SVS inhibited the action of atractyloside on energy metabolism including glycolysis, glycogenolysis, gluconeogenesis and O_2 uptake in the isolated perfused rat liver (Ishü and Bracht, 1986) However, it was suggested that the site of SVS's action on atractyloside is located outside plasma membrane. In contrary, Yamamoto and his colleugues (1985) found no effect of SVS on O_2 uptake, gluconeogenesis in rat renal cortical tubules whereas steviol and isosteviol reduced these biochemical reactions. It is likely that, SVS is without any inhibitory influence on energy metabolism in intact cells which is contrast to the isolated organelle. This is possibly due to its structure which has dissacharide and may not permeat through the cell membrane. This suggestion has been supported by Bracht et al. (1985b). They revealed no significant permeation of erythrocyte cell membrane by SVS, even after 2 hours of incubation at 37°C. The same result was obtained by Ishü and Bracht (1986).

4.4 Antimicrobial activity of SVS

SVS was found to be a less favarable substrate for Streptococcus mutano serotypes with regard to acid production than either sucrose, glucose, or fructose.

This suggestes that it can be used in oral preparation as a sweetener to lower the incidence of dental caries (Kinghorn and Soejarto, 1985 ; Ikeda, 1982). Several studies attemp to investigate the effect of SVS on growth and metabolic activity of microrganisms commonly associated with dental caries. SVS was found to inhibit microbial growth. Even though the combination of glucose and SVS was given, moderate to marked inhibitory effect against L. plantarum and L. casei and S. mutans was appeared respectively. By these experimental evidences, it implies that SVS not only gives the great sweetness but also protects dental caries which is contrast to glucose. This may be its another special properties for human use.

4.5 Effect on cardiopulmonary system

Base on the work carried out in Brazil, tea prepared from Stevia leaves resulted a discrete lowering of the systolic and diastolic blood pressure around 9.5% in 18 heatlhy human subjected to Stevia daily for 30 days (Kinghorn and Soejarto, 1985). Humboldt and Boeckh (1978) reported that SVS-treated rats displayed a significant reduction of mean arterial pressure and diuresis and shortening of the duration of electrical systole, suggesting a positive inotropic effect. However, no significant changes of respiration, BP, cardiogram and body temperature were noted in male rabbits given 15 g/kg.BW of SVS (69% purity) orally within 1 hour. The fall of BP and HR in associated with the rise of respiration were exhibited when 0.4 g/kg.BW of SVS was given intravenously (Kurahashi et al., 1982). It was suggested by several studies that the initial drop of BP and HR seems to be recovered within 1 hour following intravenous infusion of SVS. Recently, intravenous SVS administration has been found to produce hypotension in along with diuresis and

natriuresis (Melis and Sainati, 1991a, b ; Melis, 1992b). Moreover, intravenous infusion of SVS also reduced BP in hypertensive rat (Melis, 1992b).

4.6 Effect of SVS on renal function

The effect of SVS on renal function has been evaluated in a few years. In rat, the renal effect of SVS is varied depending on the dose, route and duration of administration. 4.1 g/kg.BW of SVS orally in the period of 6 hours caused the decline of urine excretion, slightly elevation of plasma BUN and creatinine as well as the damage of proximal tubule (Panichkul et al., 1988). The elevation of urinary enzymes which are the marker of proximal tubular damage such as N-acetyl-β-D-glucuronidase (NAG) and γ-glutamyl transpeptidase (γ--GTP), were detected in rat treated with 1.5 g/kg.BW of SVS subcutanously (Toskulkao et al, 1994). Disruption of microvilli and nuclear dysfunction was also found in proximal convoluted tubular cells. However, no significant alteration in the animal subjected to the lower dose as previously stated in its toxicological evaluation. More recently, Melis (1995) has demonstrated that chronic administration of Stevia extract induced hypotension and diuresis. Similarry, Melis and Sainati (1991a,b) found the diuretic and natriuretic effect without change of GFR except increase RPF in rat treated with intravenous infusion of SVS. The same results were obtained in hypertensive rat but the rise of both GFR and RPF was apparent (Melis, 1992b). However, the real site and mechanism of its action on renal function has not yet clarified. Melis and Sainati (1991a) suggested that prostaglandin may inpart participate in renal effect of SVS. Furthermore, they (1991b) proposed that SVS may act as a calcium channel blocker as verapamil.

Renal tubular handling of substances

1. Renal tubular handling of Na^+ and Cl^-

The reabsorption of Na^+ and Cl^- play a major role to regulate body elelctrolyte and H_2O homeostasis. The quantity of Na^+ excreted is adjusted by the kidney to meet the salt intake in such a way that Na^+ concentration and thus extracellular volume remain constant. Na^+ reabsorption is mainly a primary active process and depends on the Na^+, K^+ ATPase pump in the basolateral membrane whereas Cl^- reabsorption may be active or passive, depending on nephron segments, but in either case most Cl^- reabsorption is coupled to active reabsorption of Na^+ (Vander, 1995;Ganong, 1995).

1.1. Lithium clearance (C_{Li})

Proximal tubule (including both convoluted and straight portions) is the site of greatest Na^+ ,Cl^- and H_2O reabsorption. Approximately 65% of the filtered Na^+ and H_2O are reabsorbed at this site. Clinical method for measuring proximal and distal tubular reabsorption of Na^+ and H_2O have not been exactly possible because there has no suitable marker. The common technique used today to evaluate proximal and distal tubular reabsorption of substance is micropuncture. Notwithstanding, this intervention necessitates that the animals are anesthetized and laparotomized. Moreover, the micropuncture technique only gives access to the superficial nephrons not to the deep nephrons. Finally, ethical consideration forbid its use in humans. Therefore it is not possible to correlate human disease process with reabsorption of Na^+ and H_2O in different nephron segments. For some years, the discovery of C_{Li} helps to meet the method for determination of proximal delivery of Na^+ and H_2O on

the assumption that lithium ion (Li^+) are reabsorbed throughout the proximal tubule in the same proportion as Na^+ and H_2O, but no Li^+ reabsorption or secretion occurs in distal nephron. (Thomsen, 1984 ; 1990). This technique has several advantages comparing to micropuncture method, for example, can be used both conscious and anethetized situation, easily to perform, it can be used in human, and not require special technique.

Li^+ is not normally present in body fluid in significant amounts. It belongs to the group of alkali cation (group 1A of the the periodic table) which includes Na^+ and K^+. Li^+ may be passively transported through Na^+ channel or actively reabsorbed in the same manner as Na^+ transport process (Holstein-Rathlou, 1990). The assumption that Li^+ is reabsorbed only at proximal tubule and no measurable amount reabsorbed at distal nephron has been supported by several studies. Hayslett and Kashgarian (1979), using micropuncture technique, shew that Li^+ was reabsorbed in the proximal tubule in the same extent as Na^+ and H_2O, the $(TF/P)_{Li}$ ratio being 1.0 at the end of proximal convoluted tubules, and no Li^+ reabsorption took place in distal convoluted tubule and collecting ducts. The consistent result was obtained by Shirleg et al(1983). Furthermore, linear relationship between fractional reabsorption of Li^+ versus fractional reabsorption of tubular fluid in proximal tubule was shown by C_{Li} method, micropuncture method and occlusion time-transit time method (Thomsen et al., 1981). Study on humans and animals undergoing maximal water diuresis different diuretics treatment also showed the high correlation ($\gamma = 0.94$) between C_{Li} and urine flow rate during maximum water diuresis, and between fractional lithium excretion and water excretion ($\gamma=0.84$) (Thomsen and Leyssac,1986a;Thomsen, 1990). Several

lines of evidences also supported the above assumptions (Atherton et al., 1990 ; Kirchner, 1990). Figure 2.2 demonstrates the percentage of Li^+, Na^+ and H_2O reabsorption along renal tubule. C_{Li} technique has been widely used to determine proximal delivery of Na^+ and H_2O both in human and animal in various circumstances (Christensen et al., 1986). From all of the above evidences indicate that C_{Li} method is suitable for determining proximal delivery of Na^+ and H_2O. It has been shown that SVS increased renal glucose excretion (Melis, 1992a ; Toskulkao et al, 1994). It has been known that glucose is predominantly reabsorbed at priximal tubule, and Na^+-dependent. It is possible that SVS inhibits proximal tubular Na^+ reabsorption, resulting natriuresis. C_{Li} method is, therefore, used to evaluate this possibility.

By the assumption that Li^+ must be reabsorbed at proximal tubule in the same magnitude of Na^+ and H_2O reabsorption, that is

$$TF_{Li} = P_{Li}$$

TF_{Li}, P_{Li} = Li^+ concentration in proximal tubular fluid and plasma respectively

By the assumption that Li^+ is not reabsorbed or secreted beyon proximal tubule, so the amount of Li^+ delivered from straight proximal tubules (TF_{Li} x V_{prox}) is equal to the amount excreted in urine (U_{Li} x V). V, V_{prox} are urine flow and urine flow delivered from proximal tubule.

$$\text{Consequently} \quad C_{Li} = \frac{U_{Li} \times V}{P_{Li}}$$

$$C_{Li} = \frac{TF_{Li} \times V_{prox}}{P_{Li}}$$

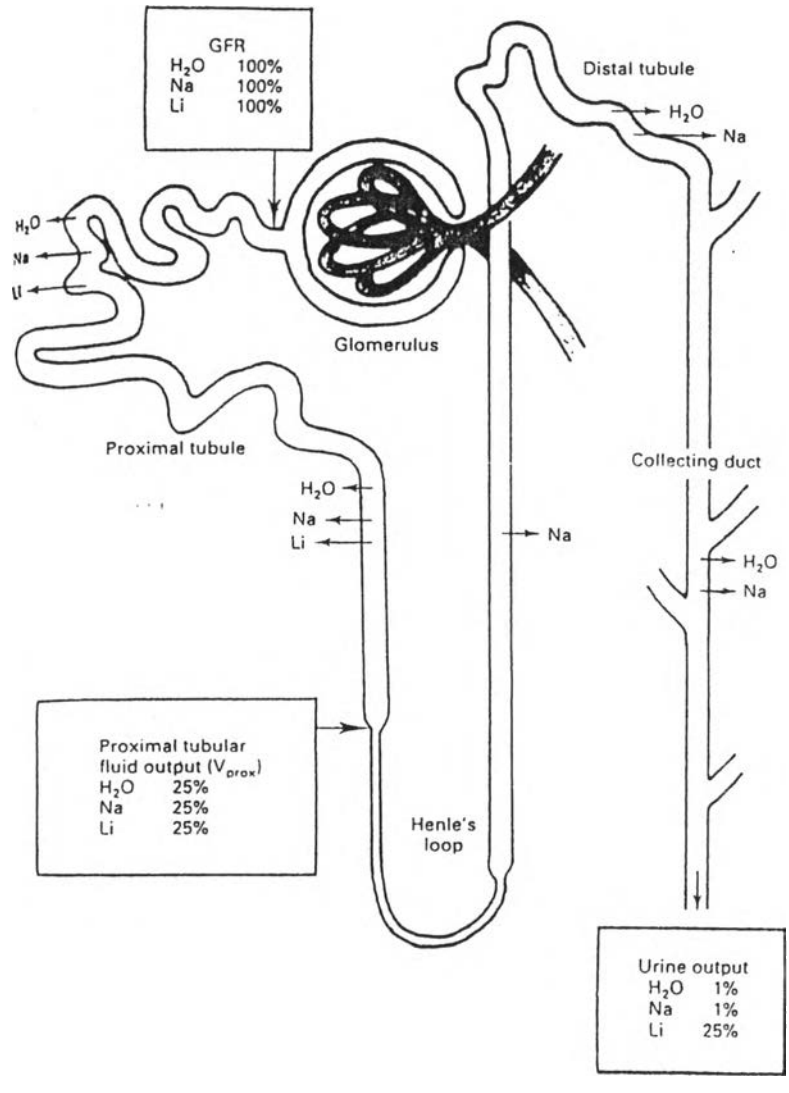

Fig. 2.2 Diagram showing a nephron with its different segments. For water, Na^+, and lithium, the percentages of the filtered load which is delivered from the proximal tubules to the loop of Henle and urine are indicated. (Thonsen, 1990)

Therefore $C_{Li} = V_{prox}$

The delivery of Na^+ from proximal tubules is expressed as C_{Naprox} which is given by $(TF_{Na} \times V_{prox})/P_{Na}$ where TF_{Na} and P_{Na} are the concentration of Na^+ in proximal tubular fluid and plasma respectively. Since $TF_{Na} = P_{Na}$ in the proximal tubules, therefore $C_{Naprox} = V_{prox}$. That is,

$$C_{Li} = C_{Naprox} = V_{prox}$$

Thus, C_{Li} can be used to estimated proximal delivery of Na^+ and H_2O. Although C_{Li} is suitable for this purpose, some precautions should be in mind. First, Li^+ was indicated to be reabsorbed at distal nephron in animals treated with low Na^+ diet and possibly low K^+ diet, resulting the lower C_{Li} and fall in urine/plasma Li^+ concentration ratio (U/P_{Li}) to below 1.0. (Thomsen and Leyssac, 1986a, 1986b; Atherton et al., 1990). By this situation, C_{Li} is not further suitable for determining proximal delivery of Na^+ and H_2O. Second, the amount of Li^+ administered to animal or human should be meet the plasma concentration of Li^+ in average 0.2-0.4 mEq/L. At high plasma Li^+ level (2-5 mEq/L), the effect of arginine vasopressin (AVP) was impaired and then caused diuresis and natriuresis (Singer et al., 1972; Galla et al., 1975). It was suggested that high level of Li^+ inhibited action of AVP, but not c-AMP production (Singer et al., 1972).

From C_{Li} study, reabsorption of Na^+ and H_2O at proximal tubules and distal nephron can be estimated as following equation.

reabsorption of H_2O in proximal tubules = GFR - C_{Li}

reabsorption of Na^+ in proximal tubules = $(GFR - C_{Li}) \times P_{Na}$

reabsorption of H_2O distally = $C_{Li} - V$

reabsorption of Na^+ distally = $(C_{Li} - C_{Na}) \times P_{Na}$

the urinary excretions of Na^+ and H_2O expressed as fractions of amount delivered from proximal tubules are given by C_{Na}/C_{Li} and V/C_{Li} respectively.

1.2 Renal regulation of Na^+ reabsorption

In normal situation, urinary Na^+ excretion is reflexly increased when there is Na^+ excess in body, and decreased when body Na^+ is deficit. These mechanisms are necessary to maintain total body Na^+ in steady level despite a wide range of Na^+ intake or loss. The regulation of body Na^+ depends on the balance between Na^+ input which mostly comes from food, and Na^+ output that is mainly excreted by kidney. Thus, the regulation of renal handling of Na^+ is the essential process on body Na^+ regulation.

1.2.1 Glomerulotubular balance

Glomerulotubular balance describes a fundamental property of the kidney whereby tubular reabsorption is adjusted in proportional to glomerular filtration. Several lines of studies demonstrated that the absolute tubular Na^- reabsorption was closely correlated in proportional to the alterations of GFR (Vander,1995;Wilcox et al,1992) Glomerulotubular balance is suggested to be the second line of defense mechanism preventing large changes of Na+ excretion. The process of glomerulotubular balance is completely intrarenal phenomenon because it does not require external renal or hormonal input and can be exhibited in isolated kidney. There is the question whether tubular Na^+ reabsorption relates to the filtered load of

Na^+ which is the product of P_{Na} and GFR, or specifically to GFR. Although controversial results have been derived, the conclusion indicates that tubular Na^+ reabsorption is closly related to GFR and not to the filtered load of Na^+ (Wilcox et al., 1992).

1.2.2. Renal enzyme Na^+, K^+ ATPase

The capacity of the tubular cells to reabsorb Na^+ depends on several factors, and one of the most important is the activity of basolateral Na^+, K^+ ATPase. This enzyme helps to pump Na^+ out of the cell in exchange with K^+. Na^+, K^+ ATPase activity is not distributed all along nephron. Indeed, nephron segments which display a high capacity of Na^+ reabsorption such as TALH, proximal tubule and distal convoluted tubule, have the highest Na^+, K^+ ATPase enzyme per tubular length unit (Doucet, 1992 ; Gullan and Mandel, 1992.) The abundant hormones and neuromediators have been proposed to influence on the number and activity of Na^+, K^+ ATPase, for example ; dopamine, thyroid hormone, norepinephrine, aldosterone;etc. (Doucet, 1992 ; Bertorello and Katz, 1993). The nephron sites and mechanisms of action of these hormone on enzyme activity are different. Aldosterone, a potent antinatriuretic hormone, acts mainly at connecting tubule where it increases Na^+, K^+ ATPase activity through induction of specific protein synthesis (Doucet, 1992). Angiotensin II and α_1 -adrenergic agonist are known to stimulate proximal tubular Na^+ reabsorption, probably through activation of phospholipase-C (PLC) or adenylate cyclase (Doucet, 1992). Prostaglandin, especially E_2 series, produces natriuresis by directly binding to receptor at collecting tubule, in addition to the interferance on the AVP activity (Cohen-Luria et al., 1993). PGE_2 directly

reduces the number of Na^+, K^+ ATPase active units through the activation of protein kinase A (PKA) and phospholipase A_2 (PLA_2). Bertorello and Katz (1993) proposed intracellular events linking between protein kinase and regulation enzyme Na^+, K^+ ATPase.

SVS infusion has been shown to induce natriuresis, and this action was partly prostaglandin-dependent (Melis and Sainati, 1991a). However, It has not yet known whether SVS affects on renal enzyme Na^+, K^+ ATPase.

1.2.3 Renal mitochondrial function

We have already discussed the significant role of Na^+, K^+ ATPase on renal tubular function. On the other hand, the activity of this enzyme is largly required energy source supplied by mitochondria. The number of studies has indicated that there is tight coupling between active transport and mitochondrial oxidative metabolism. Addition of mitochondrial metabolic inhibitors, rotenone and antimycin A, resulted the reduction of oxygen consumption (V_{O2}) and cellular ATP content in suspension of renal cortical tubule (Gullans et al., 1982). This occured in associated with the reduction of glucose and phosphate reabsorption in isolated proximal convoluted tubule. In the same manner, Soltoff and Mandel (1984) found that the alteration of intracellular ATP resulted in a proportional change of Na^+, K^+ ATPase in proximal tubule. A number of experiments also showed ATP dependent of enzyme Na^+, K^+ ATPase (Gullans and Mandel, 1992). Furthermore, modulation of energy metabolism by active transport or enzyme activity are also investigated. Inhibition of Na^+, K^+ ATPase with cardiac glycoside, ouabain, caused a decline of mitochondrial

respiration 25-30% whereas stimulation of enzyme produced the increase of respiration (Harris et al., 1981). The same results were achived by several studies (Soltoff and Mandel, 1992). From these experimental results, it seems to be that renal mitochondrial function and the enzyme Na^+, K^+ ATPase activity depends on each other.

Mitochondria is composed of two membranes, inner and outer membrane. Outer membrane is a rather simple membrane with little enzyme and transport functions, whereas inner membrane structure is complex and contains several important enzymes participating in electron transport and oxidative phosphorylation. The energy synthesis, ATP production, by mitochondria is a three step process. First, the oxidation of metabolic substrates, such as pyruvate and fatty acid, to produce reducing equivalent, NADH or $FADH_2$. The second step is the transfer of electrons along the respiratory chain from NADH and $FADH_2$ to O_2, coupled to the generation of a transmembrane electrochemical proton (H^+) gradient. Finally, the diffusion of H^+ through transmembrane F_0F_1 -ATPase to catalyze the synthesis of ATP from ADP and P_i.

The oxidation of metabolic substrate to form CO_2 is coupled to the production of NADH and $FADH_2$ from NAD^+ and FADH. The principle source of reducing equivalent (NADH and $FADH_2$) comes from tricarboxylic acid cycle (TCA cycle) which is localized within mitochondria. After reducing equivalent production in cellular cytoplasm, the transfer of electron from these reducing equivalent to O_2 along respiratory chain will be taken place. Inside inner mitochondrial membrane, electron

transport chain is arranged in a sequential pattern (Fig. 2.3), and is comprised of three large membrane-spanning multimeric protein complex and two smaller mobile protein complex. The oxidation of NADH to NAD^+ generates one proton and two electrons which are transfered to the first protein complex, NAD : ubiquinone oxidoreductase. From this protein complex the electrons are transfered to ubiquinone (UQ) which is also known as coenzyme Q (CoQ). Electrons are further transfered to the second complex protein, ubiquinol : cytochrome-C oxidoreductase, and to cytochrome C (Cyt C). The last step of electron transfer is carried to the third protein complex, cytochron C oxidase, and then to O_2 to form H_2O from $½O_2 + 2H^+$. When $FADH_2$ acts as electron donor, the electrons are donated directly to UQ, thereby bypassing the first protein complex. Some studies classify the site of electron transfer into site I, II and III which is corresponding to all three protein complex. The proton donated in various steps of protein complex provides the transmembrane proton electrochemical gradient favoring the entry of proton into mitochondria. This generates the energy to synthesize ATP within mitochondria by activation of F_0F_1 -type H^+-ATPase or ATP synthetase.

Mitochondrial respiration is often measured to assess the rate of ATP production and O_2 comsumption, and often term P/O ratio. It has been generally accepted that P/O ratio is normally to be 3 for NADH-coupled substrate, such as β-hydroxy butyrate, glutamate ; because 3 moles of ATP are formed per ½ mole of O_2 consumed. For $FADH_2$-couple substrate like succinate, P/O ratio is 2 because only 2 mole of ATP are generated. Mitochondrial respiration is tightly coupled to ATP

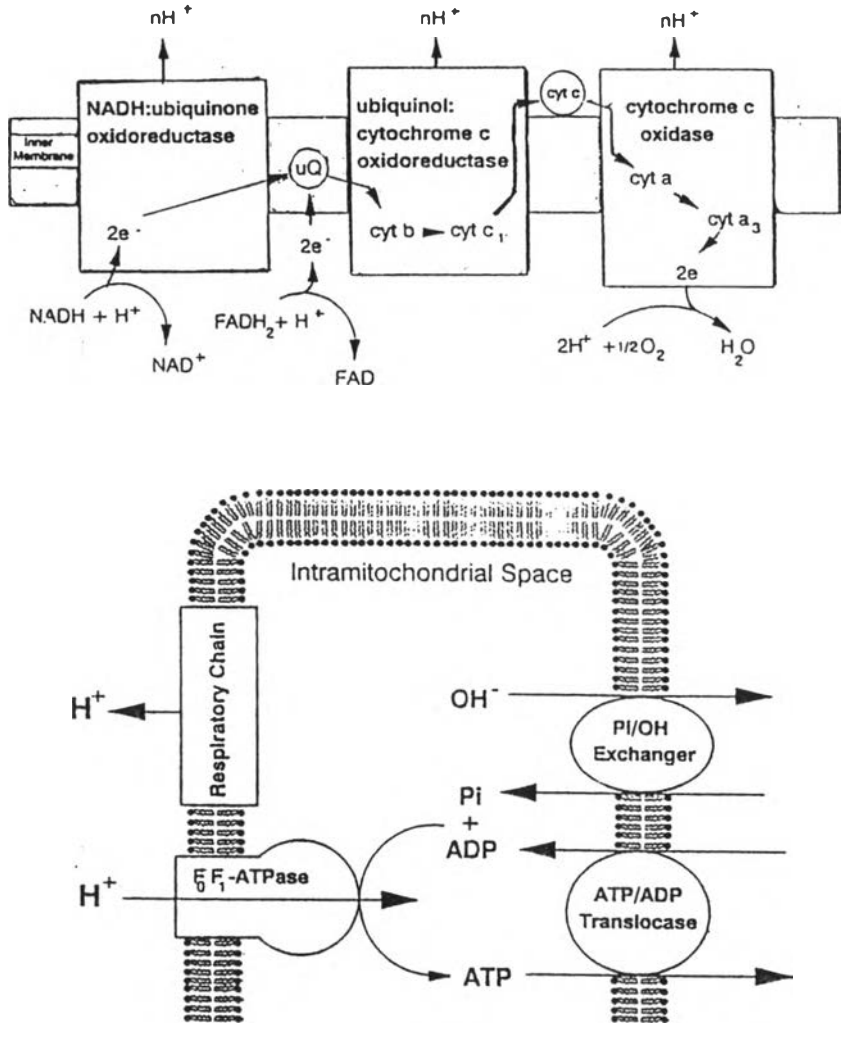

Fig. 2.3 Schematic representation of the event in mitochondrial respiratory chain (upper panel), and mechanisms of mitochondrial ATP synthesis (lower panel). (Gullans and Mandle, 1992)

synthesis, and this relationship has been called respiratory control ratio (RCR). RCR is the ratio between V_{O2} during state 3 to V_{O2} in state 4 respiration.

It was demonstrated that SVS reduced mitochondrial activity in isolated rat liver (Bracht et al, 1985a). It also enhanced urinary Na^+ excretion without change of GFR (Melis and Sainati, 1991b). Therefore, direct tubular effect of SVS, such as on renal mitochondrial function, is possible. It is, however, not known whether SVS affects on renal mitochondrial function. It is possible that SVS might reduce renal mitochondrial function, resulting natriuresis.

1.2.4. Angiotensin II (A_{II})

The octapeptide angiotensin II (A_{II}) is produced from angiotensin I liberated by the action of renin from kidney on circulating angiotensinogen. Renin and angiotensinogen can be synthesized by several tissues including brain, adrenal gland, lung, heart, reproductive system, etc. (Campbell, 1987 ; Dzau et al., 1987 ; Mene and Dunn, 1992 ; Johnston et al, 1993). Two distinct types of receptors termed AT_1 and AT_2 have recently been identified. They appears in a wide variety of tissues, including vascular smooth muscle cell (VSMC), adrenal cortex, kidney, uterus and brain (Reid, 1995). Most of the known actions of A_{II} are mediated by the AT_1 receptor. A_{II} plays an important role to regulate both BP and renal function. A_{II} has a generalized rapid (10-15 seconds) vasoconstrictor actions (Schaller et al., 1985; Sharif et al.,1992) which is approximately 40 times more potent than NE on a molar basis. Application of A_{II} receptor antagonist or angiotensin converting enzyme inhibitors significantly reduced BP in not only normal but also hypertensive animal (Mattson

and Roman, 1991; Huang et al., 1992). This indicates the significant role of endogenous A_{II} to control BP. The effect of A_{II} is also related to body fluid regulation which affects on BP. However, this effect has only a significant role in long term regulation of BP not in rapid regulation (Douglas, 1985).

For several years, a number of investigations have provided more informations to demonstrate various effects of A_{II} to regulate renal function. The effect of A_{II} on renal hemodynamics has been controversial, especially on glomerular filtration rate (GFR) depending dose administered, animal condition, route of delivery, etc.(Ichikawa and Harris, 1991). However, most observations have established that A_{II} acutely reduces renal blood flow (RBF) with little or no change of GFR, resulting in an increase of filtration fraction(FF) (Johnston et al, 1992 ; Schramek et al, 1995). This implies that A_{II} prefers constrict efferent arteriole rather than afferent arterioles. This can be supported by micropuncture study (Mene and Dunn, 1992). The predominant effect of A_{II} on efferent arterioles helps to preserve glomerular capillary pressure, and then GFR can be maintained in several situations.

The renal tubular reabsorptive effect of A_{II} has been studied in more details. A_{II} directly regulate water and solute transport independently glomerular hemodynamics (Schustler et al, 1984 ; Cogan,1990). Schusler et al (1984) demonstrated that A_{II} (10^{-11} and 10^{-10} M) increased Na^+ and volume absorption in microperfused, subcortical rabbit proximal convoluted tubule. In contrast higher A_{II} concentration (10^{-8} and 10^{-6} M) inhibited Na^+ transport. Inhibition of endogenous A_{II} action by A_{II} receptor antagonist or converting enzyme inhibitors reduced fractional

Na^+ and H_2O reabsorption (Thomas et al., 1992). This indicates that endogenous A_{II} has a regulatory role on Na^+ and fluid reabsorption.

SVS has been shown to produce hypotension, renal vasodilation as well as natriuresis (Melis, 1992b). Endogenous A_{II} has indicated to have a regulatory role on both blood pressure and renal function as discussed above. Therefore, it is possible that SVS inhibit endogenous A_{II} release to exert various effects.

1.2.5. Aldosterone

The most important hormone to control Na^+ reabsorption is aldosterone, the hormone produced by adrenal cortex. In the presence of large amounts of aldosterone, almost the remaing tubular Na^+ from the upper part of nephron is reabsorbed so that nearly none of Na^+ enteres the urine (Guyton, 1996). On the other hand, in the absence of aldosterone, almost all of the Na^+ leaving to distal tubule is not reabsorbed and then pass into the urine.

Aldosterone exhibits its effect by combinding with intracellular receptors in nucleus of principle cell of connecting tubules and collecting ducts by synthesizing mRNA which then mediates translation of specific proteins. Various theories have suggested that at least one of the synthesized protein is Na^+ channels in the luninal membrane. This intern allows greater entry of Na^+ from lumen to tubular cells. In addition, other newly synthesized proteins includes of enzyme Na^+, K^+ ATPase localized at basolateral side. Ordinarily, aldosterone's action on Na^+ reabsorption requires time, at least 90-180 min, for newly protein synthesis (Reif et al, 1986; Guyton, 1996). From the above information, aldosterone plays the significant role to

regulate renal Na^+ reabsorption. The natriuretic action of SVS might be the inhibition on aldosterone release or action.

1.2.6. Sympathetic control

Autonomic nervous system (ANS) plays an important role for rapid control of BP beginning within seconds. Its effect is brought about by actions on 2 classes of receptors, α- and β-adrenergic receptors. Single α-adrenergic blockade caused pronounced hypotension rapidly in concious rat (Schaller et al., 1985) indicating that NE alone plays a significant role for rapid BP control. Although sympathetic nerve has significant role in control of BP, it also affects on renal function. It is now generally agreed that under physiological condition, basal renal nerve activity is too low to influence renal hemodynamics because surgical or pharmacological renal denervation has no effect on RBF (Kopp and Dibona, 1992). But a reflexly induced increase in sympathetic out flow such as the reduction of BP results both afferent and efferent arteriolar vasoconstriction, and then decreases RBF and GFR (Vander, 1995 ; Kopp and Dibona, 1992). Exogenous NE administration to isolated perfused rat kidney caused the markedly reduction of both RBF and GFR (Baines and Ho, 1990), implying its potent renal vasoconstrictor effect.

Sympathetic nerve contributes not only renal hemodynamics but also tubular function. Renal sympathetic nerve has been shown to stimulate Na^+, Cl^- and H_2O reabsorption. Renal denervation caused natriuresis and diuresis in the absence changes of renal hemodynamics (Kopp and Dibona, 1992). Low frequency stimulation of renal nerve decreased urinary excretion of Na^+ and urine flow without

alteration of BP, RBF and GFR (Dibona, 1985) indicating its direct tubular effect. Renal nerve or NE exerts its actions on Na^+ reabsorption by direct binding to α_1-adrenergic receptors which mostly presence at proximal tubule (Dibona, 1985 ;Garvin, 1992). It is possible that SVS induced hypotension and alteration of renal function by inhibiting NE release.

1.2.7 Arginine vasopressin (AVP) or antidiuretic hormone (ADH)

AVP is liberated from posterior pituitary in response to the reduction of extracellar fluid volume, plasma osmolarlity and BP. There are at least 3 kinds of AVP receptors, V_{1A} , V_{1B} and V_2. They are all G-protein coupled. (Kirk and Schafer,1992 ; Ganong, 1995). Large dose of AVP elevates BP in the dose-dependent manner (Crofton et al., 1986; Wang et al., 1993). Inhibition of AVP receptors by V_{1A} receptor antagonist had no significant effect on BP but reduced cardiac output (McNeill, 1983 ; Laycock and Lightman,1989 ; Schaller et al., 1985). This implies that amount of endogenous AVP in the circulation of normal individuals does not normally influence on BP. However, in the condition that normal baroreceptor reflex mechanisms are disturbed like baroreceptor-denervated dog, hypertensive rat ; the vasoconstrictor activity of AVP is clearly expressed and the elevation of BP will be taken place (Montani et al., 1980; McNeill, 1983). It was found that AVP plays a role to maintain BP in some situations such as hemorrhage and anesthetized, surgically stressed rats (Pang, 1983 ; Ganong, 1995). Therefore, it is very likely that AVP indeed becomes an important substance for BP regulation in more prolonged states of circulatory debility but not essential for rapid regulation or in normal individual.

Although AVP has influnce on BP, its major role is the regulation of plasma osmolality and volume by enhancement H_2O and Na^+ reabsorption at collecting tubule and thick ascending limb of loop of Henle (TALH) (Reeves and Andreoli, 1992 ; Vander, 1995). These processes are mediated through V_2 receptors to produce cAMP, resulting the elevation of protein channels and special structural changes of endogenous protein channels to increase Na^+ and H_2O permeability (Nielsen et al., 1993 ; Reeves and Andreoli, 1992). Endogenous deprivation of AVP like in Brattleboro with hereditary diabetes insipidus caused the suppression of urine osmolality, GFR, RBF as well as raised urine flow (Gellai et al., 1984). SVS both infusion and feeding has been shown to induce diuresis and natriuresis (Melis, 1992a ; 1995). This action might mediate through the inhibition of AVP release.

1.2.8. Prostaglandins (PGs)

PGs are unsaturated fatty acids that arise by oxygenation and cyclization of arachidonic acid in many different types of tissues. PGs exerts a wide variety actions on numerous biologic system which participates to several physiologic and pathologic phenomena. (Despopoulos and Silbernagl, 1991 ; Hecker et al., 1995). The role of PGs in BP regulation has been widely studied. Some classes of PGs ; PGE_2, PGI_2, have vasodilator effect via activation of adenyl cyclase whereas the other, $PGF_{2\alpha}$, TXA_2 have vasoconstrictor action via formation of IP3 (Weksler, 1984 ; Hecker et al., 1995). The administration of indomethacin, an inhibitor of PGs biosynthesis, elevated BP in human and experimental animal (Salazar et al.,1995 ; Zambraski, 1995), and increased pressor response to NE and A_{II} (Nasjletti and Malik, 1981,1982). PGs attenuated vascular reactivity to pressor hormone, reduce the release of NE from

sympathetic nerve, and stimulate renin-angiotensin-aldosterone release (Lippton et al, 1988; Hall and Brands,1992). It seems likely that endogenous PGs may contribute to BP regulation by opposing pressor effect and/or release of endogenous vasoconstrictor agents.

It has been considered that endogenous PGs had no influence on renal hemodynamics in normal physiological condition (Hart and Lifschitz, 1987 ; Leicester, 1992). Rather, the effect of endogenous PGs on renal hemodynamics depends on the basal state of the animals and on the extent to which other neurohumoral vasoactive mechanisms are activated (Baer, 1988 ; Zambraski, 1995). The administration of cycloxygenase inhibitors to Na^+ deprived animals, renal ischemia, surgical stress or hemorrhagic hypotension produced the severe reduction of RBF and GFR (Dibona, 1986b; Zambraski, 1995). When renal nerve is stimulated or vasoconstrictor hormones, eg.NE or A_{II}, are present, the endogenous PGs act to couteract and limit the decrease in RBF and GFR (Hart and Lifschitz, 1987 ; Vander, 1995).

The influence of PGs on renal tubular function has been investigated in more details. Both PGE_2 and PGI_2 were found to be natriuretic and diuretic agents whereas $PGF_{2\alpha}$ was without effect (Hart and Lifschitz, 1987 ; Mene and Dunn, 1992). Several evidences showed that PGE_2 directly inhibits the reabsorption of NaCl in the ascending limb of Henle and reduces H_2O reabsorption along collecting tubule (Raymond and Lifschitz, 1986 ; Mene and Dunn, 1992). Studies on microperfusion of isolated rabbit tubules have shown an inhibitory effect of PGE_2 on Na^+ transport

(Hebert et al., 1991 ; Ling et al., 1992). On the other hand, H_2O reabsortive effect of PGs have been suggested to be inhibition of AVP-stimulated adenylate cyclase (Raymond and Lifschitz, 1986 ; Mene and Dunn, 1992).

The effect of SVS infusion on renal function and blood pressure has been partly reversed using indomethacin treatment (Melis and Sainati, 1991a), indicating the participation of prostaglandin.

1.2.9 Nitric oxide (NO)

Several years ago, a chance observation led to the discovery that endothelium also plays a key role in vasodilation. Various stimuli have been elucidated to act on endothelial cells to produce endothelium-derived relaxing factors, a substance that has now been identified as NO. NO is synthesized from amino acid L-arginine by the catalization of enzyme nitric oxide synthase (NOS) (Ignarro, 1990; Bachmann and Mundel, 1994 ;Ganong,1995). Figure2.4 illustrates the mechanism of NO biosynthesis. NO has been indicated to participate several functions including control of vascular tone, anti-platelet aggregation, cytotoxic activity and inflamatory response (Moncada et al., 1991 ; Bachmann and Mundel,1994). The physiological role of NO in BP regulation and renal functions has been investigated using various derviatives of arginine to inhibit NOS, for example, L-NAME, L-NMMA, L-NAA. Inhibition of NO synthesis raised BP in accompany with the reduction of HR and CO both in human and experimental animal (Manning et al., 1994; Widdop et al., 1992; Haynes et al., 1993). This indicates that tonic release of NO is necessary to maintain normal BP.

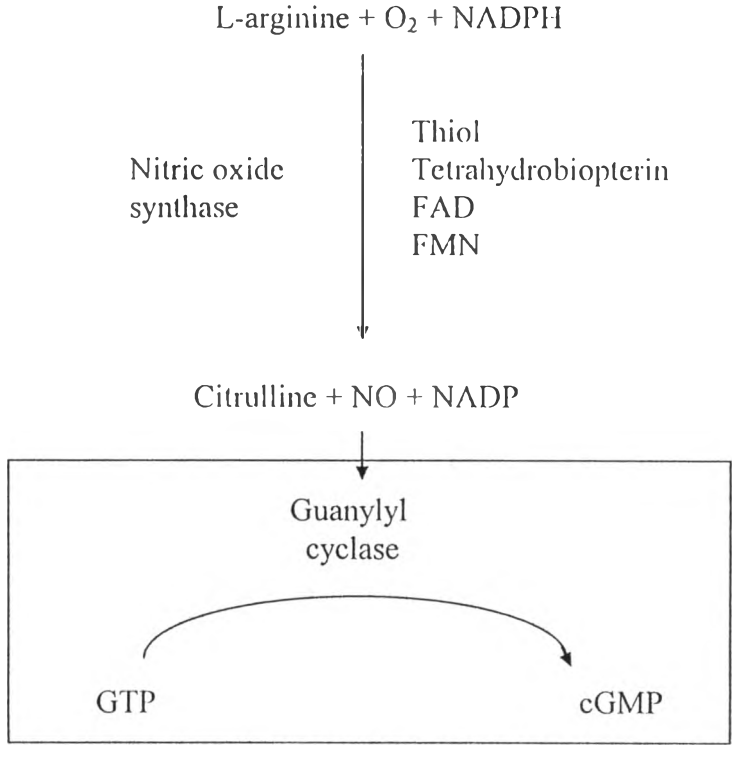

Fig. 2.4 Synthesis of nitric oxide (NO) from arginine and its action via guanylate cyclase to increase intracellular cyclic GMP (cGMP). The synthesis requires NADPH, Thiol, tetrahydrobiopterin, FAD, and FMN as cofactor. (Ganong, 1995)

More recently, the effects of NO on renal function have been extensively studied. Inhibition of endogenous NO production caused the reduction of RBF and GFR in associated with lowered Na^+ and glucose reabsorption in isolated perfused rat kidney (Radermacher et al., 1992). The same results were obtained in concious animal (Manning et al., 1994) and micropuncture study (Siragy et al., 1992 ; Nakamura et al., 1993). These indicates that endogenous NO is normally regulate renal hemodynamics and tubular functions. The effects of several agents like acetylcholine and bradykinin on regulation of BP and renal functions, has been suggested to be NO dependent (Moncada et al,1991; Ruilope et al., 1994 ; Bachman and Mundel, 1994).

NO was proposed to participate in tubuloglomerular feedback. It was speculated that an increase in intracellular Ca^{2+} released through the action of the $Na^+/K^+/2$ Cl cotransport process may link tubular fluid reabsorption to an activation of the Ca^{2+}-depedent constitutive nitroc oxide synthase I.(Bachmann and Mundel, 1994). Moreover, macular densa was suggested to produce NO which intern modulates the afferent arteriolar constriction induced by high concentration of NaCl at the macular densa(Ito and Ren, 1993). Mesengial cells contraction plays an important role to regulate GFR. It was demonstrated that NO in co-culture experiments increased mesengial cGMP levels, A_{II} mediated mesengial contraction could be inhibited by NO (Shultz et al, 1990).

There are no evidences to support that the various actions of SVS is its direct effect or mediates via any mediators. Since the effect of NO on renal functions and

blood pressure is quite similar to that of SVS, It is possible that SVS might mediate NO release.

2. Renal tubular handling of K^+

About 50-150 mmol K^+ are taken up daily (minimum requirement 25 mMol), and around 90% are excreted in urine and 10% leaves the body in the feces. Plasma K^+ concentration (P_K) is average 3.4-5.2 mmol/L whereas 20-30 fold centration localize inside the cells. Although, extracellular K^+ accounts for only 1-2% of total, it has a great importance. It's very low level in extracellular fluid is necessary for maintenance of normal cell function , especially excitable cells like muscles and nerves. The resting membrane potentials of these cells are directly related to the ratio of intracellular and extracellular K^+ concentration (Vander, 1995 ; Despopoulos and Silbernagl, 1991).

In order to maintain the low plasma K^+ level, several mechanisms must be operated. These include of K^+ uptake into the cells by various hormone such as insulin or exchange with other cations, and K^+ secretion through renal tubules. The pivotal portion of K^+ regulation is connecting tubule and cortical collecting duct to secrete K^+. The amount of K^+ secreted here is depended on body K^+ balance. The higher the body K^+ the larger the K^+ secrete is taken place.

The regulation of renal K^+ handling is undertaken by several process. The alterations of renal hemodynamics, acid-base balance, hormone aldosterone ; etc., are all influence on K^+ excretion. (Vander,1995 ; Bullock, 1995)

3. Renal handling of glucose

The proximal tubule is the major site for reabsorption of the large quantities of organic substances including glucose. Filtered glucose is almost reabsorbed at proximal convoluted tubules, hence no or so little glucose leaves into urine (Guyton, 1996 ; Ganong, 1995). The amount reabsorbed is proportional to the amount filtered and thus to plasma glucose (P_G) until transport maximum (Tm_G). The renal glucose transport is Na^+ dependent just like as in the intestine. The energy for active transport is provided by Na^+, K^+ ATPase that pumps Na^+ out of the cell to lower intracellular Na^+ concentration. This favors Na^+ co-transport with glucose from lumen into inside the cells. Therefore, inhibition of Na^+, K^+ ATPase abolishes not only tubular Na^+ reabsorption but also glucose.

The renal plasma threshold of glucose is approximately 180-220 mg% in human. As plasma glucose is further raised, the reabsorptive capacity becomes progressively saturated until the rate of reabsorption becomes constant and maximal

m_G is around 350-375 mg/min in human, 200 mg/min in dog, and 10

.at (Robson et al., 1968 ; Sulkurt, 1984). Any further elevation of plasma

beyond renal plasma threshold is accompanied by a proportionate increase

.ıe glucose excretion.

SVS has been suggested to induce urinary glucose excretion as well as natriuresis (Melis, 1992a ; Toskulkao et al, 1994). However, the mechanism responsible to glucouria during SVS administration has not yet elucidated. It may be

due to the reduction of Na^+ reabsorption. Renal plasma threshold of glucose and Tm_G should be also determined to clarify the action of SVS on renal glucose excretion.