CHAPTER I

GENERAL INTRODUCTION

Several organs in the body play an important role to regulate body homeostasis. Kidney is one of these organs which is essential for several controls such as blood pressure regulation, body fluid and electrolytes regulation, acid-base balance. Renal function can be affected by a numerous substances. Many agents can induce natriuresis and diuresis especially various types of vasodilators such as acethycholine and prostaglandin. (Baylis et al., 1976 ; Granger and Solhaug, 1972).

Stevioside (SVS) is a major sweet constituent prepared from the leaves of plant Stevia rebaudiana or Stevia. It is accounted for approximately 6-10% in dried leaves. SVS has many properties such as highly stable, well grown in tropical area and great sweetness (250-300 times sweeter than sucrose) (Hanson and De Oliveira, 1993). SVS has been widely used to be sugar substitute in Japan. The utilization is likely to be expanded in many countries in Asia. However, very few data are available for its effects and mechanisms of actions on various physiological changes particularly in relation to renal functions. Although it has been reported that SVS can affect on a significant decrease in mean arterial pressure and diuresis (Humboldt and Boeckh ; 1978, Melis and Sainati 1991a,b).

Natriuresis and diuresis can be induced by several factors such as an elevation of glomerular filtration rate (GFR), changes of physical forces at peritubular capillaries; membrane permeability ; the number and capacity of enzyme Na^+, K^+ ATPase at

basolateral side, etc. (Doucet, 1992). Intravenous infusion of SVS has been shown to cause natriuresis and diuresis without change of GFR but increase in renal plasma flow (Melis and Sainati, 1991 a,b). The changes of physical forces and/or enzyme activity would be the possible cause of natriuresis. Sodium ion is mainly reabsorbed by active transport which is drived by Na^+, K^+ ATPase. This enzyme hydrolyzes ATP to yield energy to push and exchange between Na^+ and K^+. In the rat, the highest enzyme activity are found at proximal tubule, medullary thick ascending limb and distal convoluted tubule (Doucet, 1992). Natriuretic effect of SVS in the relation to the reduction of this enzyme activity has not yet clarified. SVS directly affect on enzyme activity and/or energy source supplied by renal mitochondria. Mitochondrial inhibitors such as rotenone and antimycin A has been shown to reduce fluid, glucose and phosphate reabsorption in isolated perfuse proximal convoluted tubule (Gullans et al., 1982). Toxicant-induced interruption of renal mitochondrial function will bring about the reduction of Na^+ and water (H_2O) reabsorption. The action of SVS has been demonstrated to inhibit mitochondrial oxidative phosphorylation in isolated rat liver (Bracht et al ,1985a,b). However, the effect of SVS on renal mitochondrial function has not yet been elucidated.

More than 80% of filtered solutes are reabsorbed at proximal tubule. Moreover, proximal tubule are claimed to be highly vulnerable to diverse nephrotoxic agents. Several lines of evidences confirm this suggestion (Guder and Ross, 1984 ; Stonard, 1990). From histopathological finding, proximal tubular damage along with oliguria was exhibited in rats subjected to SVS intubation (Deechakawan, 1992 ; Panickul et al., 1988). However, no evidences support the site of action of

intravenous infusion of SVS. Natriuresis and diuresis-induced by SVS are probably due to its effect to inhibit proximal tubular reabsorption.

Other than natriuretic and diuretic effect, SVS increased renal glucose clearance (Melis, 1992a). It is unknown how and what mechanism is. It is widely known that renal glucose reabsorption is Na^+-dependent (Deetjen et al., 1992). The declination of Na^+ reabsorption induced by SVS possibly result the reduction of glucose reabsorption. Furthermore, SVS was found to reduce glucose transport across isolated rat liver (Bracht, 1985b). Therefore, it is possible that glucouric effect of SVS may be due to direct effect of SVS on glucose transport across renal tubular cell without participation of its natriuretic effect.

Several lines of evidences have shown that SVS and aqueous extracts of *Stevia* are active in several physiological system including metabolism. Oviédo and co-worker (1970) found the decreae in blood glucose level in 25 healthy human treated with extracts of Stevia leaves orally without specifying the dose employed. Similar result was obtained by Zuzuki et al. (1972). However, the controversial results are also presented. Lee and his colleagues (1979) reported no changes in blood glucose level when crude extracts of Stevia leaves were fed to rats for 56 days, each rat consumed 0.5-1 g extract/day. The same result was obtained after feeding the rats by 7% of Stevia extracts. From the evidences mentioned earlier, there are some conflicting results about the influence of Stevi*a* extracts on blood glucose. Moreover, the real effect and mechanism of SVS's action on glucose metabolism has not yet

clarified. The glycemic effect of SVS may participate to its glucouric and natriuretic influence.

For the various effects of SVS, it may exert the direct or indirect effect via other intermediates on several organs. Melis and Sainati (1991a) reported that the hypotensive and renal effect of intravenous infusion of SVS probably depended on prostaglandins. They also demonstrated that SVS acted as verapamil, calcium antagonist, on renal function and blood pressure. However, the real mechanism of SVS's action on physiological system especially blood pressure and renal function has not yet elucidated.

Thus, the objective of the present experiment is to investigate the various physiological responses in vivo and the mechanisms of responses when administration of SVS. The experiment for the effect of SVS on renal function was carried out (chapter IV). Further examinations were manifested to clarify the site and mechanisms of natriuretic effect using lithium clearance method and evaluation of renal Na^+, K^+ ATPase and mitochondrial function (chapter V). Moreover, the mechanism responsible for the various alterations whether changes were mediated by interfering to other systems in the body, for example, prostaglandin, renin-angiotensin system, adrenergic nervous system, arginine vasopressin and nitric oxide (chapter VII). The alteration of glucose metabolism during SVS administration and mechanism of its actions was another purpose to clarify by measuring glucose turnover rate and plasma insulin level (chapter VI). Not only natriuresis and diuresis but also glycouric effect of SVS was appeared. However, the mechanism responsible

for this change has not yet elucidated. All experiments (chapter IV-VII) will be discussed in the general discussion (chapter VIII).