CHAPTER VIII

GENERAL DISCUSSION

The action of SVS has been studied for a decade especially its toxicological effects. However, the investigation particulary in relation to renal function has not been available. It has been shown from the present results (chapter IV-VII) that many bodily functions in animals given of SVS either intravenous infusion or intubation were affected, including systemic hemodynamics, renal function and glucose kinetics. Nevertheless, the mechanism of its action has not yet been elucidated. Therefore, the present study was performed to clarify the effects and mechanisms of actions of SVS on renal function and other physiological effects in relation to these variables.

The experimental results in chapter IV illustrate that the effect of SVS given either intravenous infusion or intubation affect on both blood pressure and the heart rate (HR). Administration of SVS either intravenous infusion or intubation caused hypotension but difference in magnitude. HR significantly increased in corresponding to hypotension. Intravenous infusion of SVS produced the reduction of blood pressure to the lowest level in regard to dose and time dependent manner. The lowest value of blood pressure was apparent at 5-7 min after SVS infusion, and then gradually elevated afterward. The decline of blood pressure in response to SVS infusion or intubation has been recently reported in both normotensive and hypertensive rats (Melis and Sainati ,1991a; Melis, 1992b,1995). After cessation of SVS infusion, the recovery of the blood pressure was not accomplished in rats treated

with the large (200 mg/kg.BW) and medium doses (150 ml/kg.BW). This was not due to the time effect but rather the result of SVS infusion per se, since SVS was found to remain in the circulation within 2 hours after SVS infusion (Ishü and Bracht, 1995). The reduction of blood pressure during SVS infusion was shown to be due to its vasodilator effect which was not due to the decrease of blood volume. However, this hypotension is probably due to its direct effect on vascular smooth muscle cell or mediated by an activation on various vasodilators like prostaglandin, nitric oxide (NO) and acetylcholine or inhibition the vasoconstrictors release such as norepinephrine (NE), angiotension II (A_{II}) and arginine vasopressin(AVP). It has been shown that the initial decrease of blood pressure after SVS infusion would be mediated via NO whereas prostaglandin and sympathetic adrenergic nerve participate in the latter period until the end of experiment (chapter VII). The release of NO would account for the hypotension. The response of sympathetic stimulation would be attributed to support the normalization of blood pressure and elevation of HR in the latter period of SVS infusion. Although the blood pressure was gradually increased but it could not return to the normal level. The interaction between sympathetic nerve activity and prostaglandin would be suspected. Sympathetic activation can stimulate prostaglandin release (Dibona, 1986 ; Lippton et al., 1988). Moreover, prostaglandin was shown to reduce vascular response to NE (Dibona, 1986 ; Lippton et al., 1988). Therefore, the failure of blood pressure recovery is probably the interaction of prostaglandin to overide sympathetic activity. The complete recovery of blood pressure and HR following the initial hypotension in rats pretreated with indomethacin

has been noted. It is still unclear whether prostaglandin release is the direct stimulation from SVS infusion or from sympathetic activation

It becomes clear that the actions of prostaglandin, NO and sympathetic adrenergic activity account for hypotension and tachycardia during and after SVS infusion (chapter IV and VII). However, the mechanisms contribute to hypotensive effect of SVS intubation has not yet elucidated. SVS has been shown to be decomposed by the rat intestinal microflora to steviol both in vivo and in vitro experiments (Wingard et al., 1980; Nakayama et al., 1986). Therefore, it is possible that SVS feeding exerted its hypotensive effect through steviol. Nevertheless, no experimental evidences have been available to clarify the effect of steviol on the cardiovascular function.

From the experiments in chapter IV and VII, SVS intubation for 6 hours had no significant effect on renal hemodynamics. In contrary, 30-min of SVS infusion caused the elevation of ERBF without a change in GFR, suggesting the vasodilatation in both renal afferent and efferent arterioles. It was noted that autoregulation of GFR is still intact despite hypotension. Prostaglandin and arginine vasopressin (AVP) were revealed to participate in renal vasodilating effect during SVS infusion. Pretreatment with indomethacin or AVP prevented an increase in ERBF during SVS infusion. It has been demonstrated that inhibition of endogenous or exogenous administration of AVP had no significant effect on renal hemodynamics (Söndeen and Claybaugh, 1989 ; Rose et al., 1991). Moreover, AVP pretreatment reversed ERBF during SVS infusion, and showed the similar result as pretreatment period (chapter VII). Therefore, the

recovery of renal hemodynamics by AVP should not the result from its direct action on renal vasculature. Prostaglandin was demonstrated to participate in the action of SVS infusion on blood pressure and heart rate as shown in chapter VII. It might be that AVP administration inhibit prostaglandin release or action on renal vasculatures. This possibility seems to be unlikely since AVP has been reported to stimulate prostaglandin synthesis (Walker et al., 1978 ; Zusman, 1981). Therefore, it would rather be that AVP administration would inhibit SVS-induced prostaglandin liberation or action on renal vasculature.

The effect of SVS infusion on renal vasodilatation was not presented following the first period but significant reduction of GFR was apparent after stopping infusion of the large and medium doses of SVS infusion. The mechanism responsible to this change was unknown. From histopathological examination revealed that the general congestion of glomerular capillaries was apparent in animals treated with large and medium doses of SVS infusion. This may be the possible cause of the reduction of GFR.

Urine flow rate markedly increased during the first period of SVS infusion (chapter IV, V and VII). Similar result was obtained after SVS intubation for 6 hours. Furthermore, renal excretion of Na^+, Cl^- and slightly K^+ were observed. The similar results have been reported.(Melis and Sainati, 1991a, Melis, 1995). The facilitation of H_2O and electrolyte excretion without changes of GFR and the plasma electrolytes concentration should be the result from the depression of renal tubular reabsorption. It was noted that the fractional excretion of glucose (FE_G) significantly raised in

associated with the elevation of FE_{Na} in rats treated with SVS infusion (chapter IV and VI). The rise of urinary glucose excretion along with the proximal tubular degeneration was detected in the rat subjected with subcutaneous injection of SVS for 9 hours (Toskulkao et al., 1994). It has been known that the proximal tubular glucose reabsorption is Na^+-dependent (Deetjen et al, 1992). Therefore, it is possible that natriuresis and diuresis induced by SVS infusion should be the result from the suppression of proximal tubular Na^+ reabsorption in the present study. This suggestion is supported by the clearance litium (C_{Li}) study (see chapter V). It has been estrablished that the tubular reabsorption of Li is proportional to the reabsorption of Na^+ and H_2O at proximal tubule (Thomsen et al., 1984 ; 1990). By using the C_{Li} technique, the reduction of proximal tubular Na^+ and H_2O reabsorption is related to the increase of Na^+ clearance (C_{Na}) and FE_{Na}. This correlation was remained despite the cessation of SVS infusion. Therefore, the reduction of proximal tubular Na^+ and H_2O reabsorption would be the predominant site of action of SVS infusion causing both natriuresis and diuresis during and after SVS infusion.

Several mechanisms have been proposed to be the cause of Na^+, Cl^- and H_2O excretion during SVS infusion, for example, renal Na^+, K^+ ATPase activity, renal mitochondrial activity, etc. Renal Na^+, K^+ ATPase which is the key enzyme necessary for tubular Na^+ reabsorption, has been found to present mostly at the proximal tubule in the rat (Doucet, 1992). In the kidney of rat, more than 85% of this enzyme has been localized in renal cortex (Higgins et al., 1978). Our experimental results revealed the significant reduction of renal Na^+, K^+ ATPase activity in renal cortex and whole kidney in response to 30 min of SVS infusion (chapter V). SVS may, however,

directly interfere this enzyme and/or energy supply. The state 3 respiration (ADP-stimulated respiration) and the renal mitochondrial ATP synthetase activity were reduced. This indicates that there is at least the impairment of mitochondrial oxidative phosphorylation to genarate energy supply. Thus, it is clear that 30-min of SVS infusion depresses both renal Na^+, K^+ ATPase and mitochondrial activity, and then ultimately reduces proximal tubular Na^+ reabsorption.

Although SVS infusion depresses renal Na^+, K^+ ATPase and mitochondrial activity, the magnitude of suppression was only 21 and 14% respectively whereas the reduction of proximal tubular Na^+ reabsorption was markedly increased in a high level (88%) (chapter V). This result implies that the reductions of both Na^+, K^+ ATPase and mitochondrial activity should not be the major cause of a marked natriuresis. The other factors such as the change of starling forces at the peritubular capillaries induced by the vasodilator effect of SVS may be possible (Vander,1995). Despite the absence of renal vasodilator effect of SVS in rats pretreated with indomethacin or AVP, the significant elevation of FE_{Na} was still apparent in the first 30 min period of SVS infusion (chapter VII). Moreover, SVS infusion remained induced the elevation of Na^+ excretion despite the appearance of renal vasoconstriction in rats pretreated with NE, A_{II} or L-NAME. Therefore, the alteration of starling forces at peritubular capillaries at proximal tubule would not suspect to account for the elevation of electrolytes excretion during SVS infusion. Neurohormonal alteration induced by SVS infusion to affect the renal tubular reabsorption would not be possible since animals pretreated with various drugs in the present experiment did not interfere the natriuresis in the first period of SVS infusion

(chapter VII). The direct effect of SVS infusion on renal tubular reabsorptive capacity should become the major cause of the profound electrolyte excretion in the first 30-min of SVS infusion. From the histopathological examination, proximal tubular cell impairment was shown in the renal tissue of animals treated with SVS infusion, and might result the reduction of tubular reabsorption.(Walker and Duggin, 1992)

The enhancement of urinary Na^+ and Cl^- excretion was still exhibited during the second period of SVS infusion (chapter IV, V and VII) which was coincided to the reduction of the proximal tubular Na^+ reabsorption as studied by the C_{Li} technique (chapter V). Glucosuria was also apparent which would imply the proximal tubular defect of Na^+ reabsorption. The reduction of renal Na^+, K^+ATPase and mitochondrial activity may participate. The alterations of staring forces at proximal tubules are unlikely since slight change of renal hemodynamics was produced. SVS-induced neurohormonal alteration to affect renal tubular reabsorption of Na^+ may be another possible mechanism. Only pretreatment of AVP or indomethacin reversed the natriuretic effect of SVS infusion during the second period (chapter VII). However, the mechanism underlying the recovery of reabsorption of Na^+ and Cl^- during SVS infusion in both groups is unknown. AVP and prostaglandin have been reported to affect on renal tubular Na^+ and H_2O reabsorption at the thick ascending limb of loop of Henle and the collecting tubule (Rouffignac et al., 1983 ; Baer, 1988 ; Amlyn and Ludens, 1992) not the proximal tubule. This indicates that the reversibility of the Na^+ and Cl^- excretion in both groups was not due to the effect of both drugs on proximal tubular Na^+ and Cl^- reabsorption. Therefore, the reduction of proximal tubular Na^+ reabsorption produced by SVS infusion should be still existed. Supporting to this

interpretation is that the elevation of glucose excretion was maintained in both groups during this period. Therefore, the facilitation of Na^+, Cl^- and H_2O excretion during SVS infusion should not be due to the direct inhibition of AVP and/or stimulation of prostaglandin release. Prostaglandin has been shown to affect urinary concentrating ability by modulating the effect of AVP on Na^+ and H_2O reabsorption at medullary thick ascending limb and collecting tubule (Vander, 1995 ; Roy et al., 1992; Reeves and Andreoli,1992). Prostaglandin also increased medullary blood flow whereas AVP expressed the opposite result (Baer, 1988 ; Roy et al., 1992). In addition, AVP was found to stimulate Na^+ and H_2O reabsorption at thick ascending limb and collecting tubule (Baer, 1988; Reeves and Andreoli, 1992). The action of AVP and prostaglandin on renal handling of NaCl is not only their direct effect on renal tubule but also via the alteration of medullary blood flow. From the experimental results mention earlier, it is possible that SVS directly blocked the proximal tubular Na^+ reabsorption which coincided with the stimulation of prostaglandin synthesis and/or inhibition of AVP release. However, indomethacin and AVP pretreatment had no effect on renal tubular action of SVS along with the absence change of renal hemodynamics in the first period. In contrary, the recovery of Na^+ and Cl^- excretion during the second period is relatively associated with the reduction of ERBF. It is likely that the reduction of ERBF, especially medullary blood flow by AVP and indomethacin administration, may increase tubular reabsorption of a large amount of Na^+ and Cl^- leaving from proximal tubule. In summary, the enhance of electrolyte excretion in response to SVS infusion is not directly mediated by any drugs used in the present study. The recovery

of electrolyte excretion produced by the pretreatment with AVP and indomethacin should not be due to their actions on proximal tubular effect of SVS infusion.

After cessation of SVS infusion, the rise of Na^+ and H_2O excretion was still detected although the reduction of GFR was apparent (chapter IV and V). Proximal tubule was found to be the primary site as suggested by the C_{Li} study (chapter V) and significant increase of urinary glucose excretion (chapter VI). Time effect is not the cause that leads to the alteration of GFR and Na^+ excretion (chapter IV). Glomerulotubular balance may be operated to adjust proximal tubular Na^+ reabsorption in relation to the reduction of GFR (Vander, 1995 ; Bullock, 1995a). Furthermore, the elevation of Na^+, Cl^- and H_2O excretion after cessation of SVS infusion might be the result from the action of SVS per se, since SVS has been reported to be maintained within 2 hrs after administration (Ishü and bracht, 1995).

SVS intubation for 6 hours resulted an elevation of urinary excretion of Na^+, K^+, and Cl^- without a change of GFR. This indicates the tubular effect of SVS on electrolyte reabsorption. The depression of proximal tubular reabsorption is unlikely since FE_G was not changed. Therefore, site of tubular effect of SVS should be behind proximal tubule. This effect may depend on enzyme activity, hormonal changes or direct action on tubular reabsorptive cells. As previously stated that SVS intubation could be decomposed to other substance called steviol. Steviol could inhibit mitochondrial activity in isolated rat liver (Bracht et al., 1985b), and this may present in renal mitochondrial function. However, the experimental evidence to support the actions of steviol on renal function has not yet been available.

SVS infusion had markedly effect on Na^+ and Cl^- excretion but slight or no effect on K^+ excretion (chapter IV and VII). The possible explanation should be the reduction of proximal tubular HCO_3^- and K^+ reabsorption in associated with the decrease in Na^+ reabsorption (Bullock,1995a; Vander,1995). This causes peritubular capillaries acidosis, and thereby prefers the exchange of Na^+ with H^+ rather than K^+ at distal nephron. Furthermore, H^+ -K^+ ATPase activity may also increase to get rid of the excess H^+ in peritubular capillaries. For these results urinary acidification might be expected to occured. The reduction of K^+ secretion at distal nephron along with the reduction of proximal tubular K^+ reabsorption associated with the reduction of proximal Na^+ reabsorption, finally probably leads to slight change of K^+ excretion in urine.

The profound excretion of glucose in urine during and after SVS infusion but not in SVS intubation was demonstrated in chapter VI and VII. SVS infusion caused an elevation of the plasma glucose concentration (P_G) to the level of 140-150 mg%. In contrary, SVS intubation had no effect on the P_G level (chapter VI). The hyperglycemic effect of SVS infusion was continued despite the cessation of its administration in group of the large and medium doses of infusion. No experimental evidences have been available to support the action of SVS infusion on P_G and glucose kinetics. Most studies try to explore the effect of SVS feeding on P_G level, and showed the different results depending on the experimental model, duration, dose and the experimental procedure (Lee et al., 1979, Curi et al., 1986 ; Toskulkao and Sutherawattananon,1994). The absent effect of SVS intubation on P_G in the present experiment might be that the amount and duration of SVS intubation are not enough

to induce a change of P_G . This dose and duration of SVS intubation was chosen since it could produce hypotension, natriuresis and diuresis as shown in chapter IV.

The mechanisms underlying to an elevation of the P_G level during SVS infusion is clarified by the present study. From the present study for the glucose turnover rate which presents the first estimation relating to the effect of SVS infusion indicates the reduction of glucose uptake without a change of the plasma insulin level. Accordingly, the rise of P_G level in response to SVS infusion is not due to the decline of insulin release. Tissue response to insulin is, however, still intact. It is interesting to note that despite the rise of P_G level, insulin release was unaltered, indicating the impairment of glucose stimulated-insulin release during SVS infusion. It has been suggested that SVS infusion acted as Ca^{2+} channel blocker to induce hypotension and natriuresis , and reversed by Ca^{2+} administration (Melis and Sainati, 1991b). The glucose-stimulated insulin release is dependent on the elevation of intracellular Ca^{2+} in pancreatic β-cell (Black et al, 1994). Therefore, the inhibitory action of SVS infusion on glucose-stimulated insulin release is possibly due to the reduction of Ca^{2+} uptake into pancreatic β-cell.

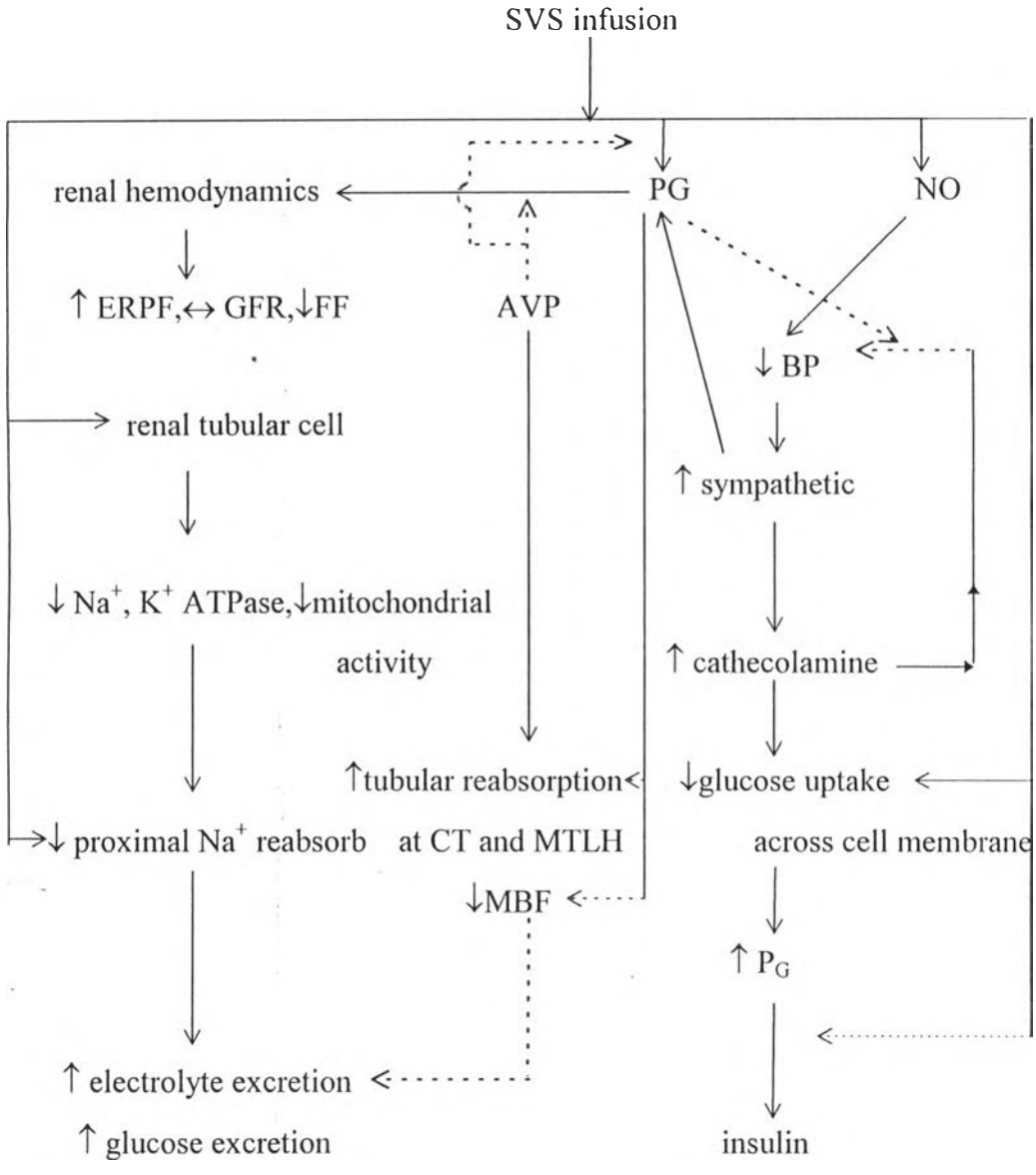
The various possible mechanisms for the effect of SVS infusion to raise plasma glucose level were proposed. The hyperglycemic appearance may be the direct or indirect effect of SVS infusion. SVS has been found to inhibit glucose transport across the cell membrane in perfused rat liver (Brach et al., 1985b ; Ishü et al., 1987). However, it is unknown whether SVS inhibits glucose uptake in intact animal since there might have several neurohormonal changes in intact animal during SVS infusion

that affect on glucose transport. As suggested previously in chapter VII that sympathetic activity, NO and prostaglandin participated in hypotension, diuresis and natriuresis during SVS infusion. These drugs were also shown to participate on the P_G level induced by SVS infusion. The P_G level could be attenuated in the second period of SVS infusion in animals pretreated with indomethacin or L-NAME or prazosin. However, the P_G level seemed to return back to the normal value during the second period in rats pretreated with a combination infusion of indomethacin and L-NAME. The mechanism that these drugs involve the hyperglycemic action of SVS infusion is not known. There might have some interactions among norepinephine, NO and prostaglandin on the hyperglycemic action of SVS infusion. After cessation of SVS infusion, the rise of P_G level was remained in the groups subjected to the large and medium doses of SVS. The mechanism responsible to this phenomenon might be similar to above mention that the action of SVS was still remained.

Although SVS infusion raised plasma glucose concentration, it is less than renal plasma threshold of glucose (chapter VI). Similary, renal tubular transport of glucose is much less than tubular transport maximum of glucose (T_{mG}). It was noted that, the increase of FE_G was associated with the reduction of proximal tubular Na^+ reabsorption (chapter V and VI). The renal tubular reabsorption of glucose is Na^+-dependent. Therefore, the significant glucosuria in both during and after SVS infusion is mainly the result from the depression of proximal tubular Na^+ reabsorption.

In conclusion, SVS infusion produced hypotension via the release of NO,and then stimulates sympathetic adrenergic activity during compensation for hypotension.

However, the compensation was not accomplished since the action of prostaglandin to overide that of sympathetic activity. Renal vasodilatation is predominantly mediated via prostaglandin. Proximal tubule is the primary site of natriuretic and diuretic effect of SVS infusion whereas SVS intubation exerts its tubular action beyond proximal tubule. The magnitude of the reduction of renal Na^+, K^+ ATPase and mitochondrial activity is too low to explain the profound natriuresis during SVS infusion. Also, the alteration of starling forces at peritubular capillaries induced by its renal vasodilator effect does not play an important role to induce natriuresis and diuresis. Direct effect of SVS infusion on tubular reabsorptive capacity is the most possibility. AVP and prostaglandin synthesis inhibition reversed its effect on Na^+ and Cl^- excretion. However, they do not interfere the inhibitory action of SVS infusion on proximal tubular reabsorption. SVS infusion raised P_G level by its effect to reduce glucose uptake across cell membrane. Sympathetic activation, NO and prostaglandin were shown to participate on hyperglycemic action of SVS infusion. SVS infusion also inhibited glucose-induced insulin release. While SVS raised plasma glucose level, SVS intubation had no any effect on plasma glucose level. The appearance of glucosuria during and after SVS infusion was primary due to the reduction of proximal tubular Na^+ reabsorption not due to the rise of plasma glucose level. All possible explanation can be summarized in the following diagram.



---- inhibition , PG= prostaglandin, BP = blood pressure, MBF= medullary blood flow

Figure 8.1 Schematic represents the proposed mechanisms of action of SVS on renal function

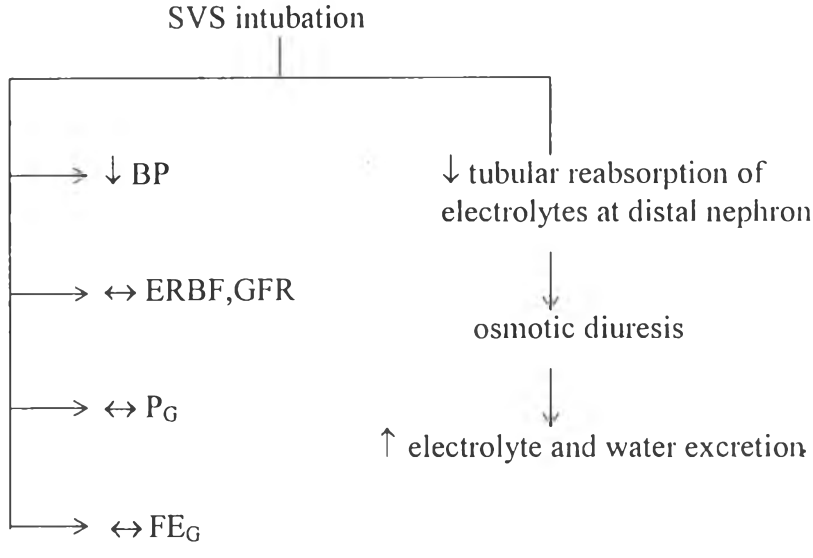


Figure 8.2 Schematic represents the of action of SVS intubation on renal function