CHAPTER 5

DISCUSSION

In the present study, brimonidine decreased mean arterial pressure (MAP) and heart rate (HR) simultaneously. When specific antidote, yohimbine was administered, the return of MAP and HR were exaggerated compared to baseline levels. Brimonidine also decreased the ratio between MAP and R-R interval. Moreover, MAP and HR were fluctuated within the first hour after brimonidine and yohimbine administrations as the increases in standard deviation of MAP (SDMAP) and HR (SDHR) in that period. In this case the statistical significance was noticed only in SDMAP. On electrocardiogram, brimonidine prolonged P-R interval. Although the significance was undetectable, Q-T interval was also extended after brimonidine administration. After yohimbine was injected, P-R and Q-T interval was diminished when compare to baseline values.

Biphasic effect on blood pressure was elucidated in many reports which studied alpha 2-adrenoceptor agonists in dogs (Pypendop and Verstegen, 2000; Kuusela et al., 2001). In these studies, after alpha 2-adrenoceptor agonists administration, systemic blood pressure became transient elevation and then reduced to the level under baseline value which maintained for several hours. Heart rate also decreased after administration and also sustained for several hours.

The mechanism of this phenomenon involved blood pressure and heart rate by both central and peripheral nervous systems. Counterbalance between the effects of central and peripheral alpha 2-adrenoceptor activation was a key to explain changes in the mean arterial pressure. The activation of central alpha 2-adrenoceptor in neurons which project from rostral ventrolateral medulla (RVML) to intermediolateral column of the spinal cord, lead to inhibit the sympathetic outflow, directly shown by the decrease in plasma norepinephrine concentration (Bock et al., 1999) heart rate, cardiac output (Pypendop and Verstegen, 2000). Thus, blood pressure was suspected to be reduced after drug treatment.

The direct effect of romifidine, another alpha 2-adrenoceptor agonist has been shown to affect peripherally the vascular smooth muscle-alpha 2-adrenoceptors, including vasoconstriction. Pypendop and Verstegen (2000) demonstrated that systemic vascular resistance increased in response to romifidine administration, which contributed the initial hypertension in conscious dogs.

Central and peripheral effects of brimonidine seem to be in opposite way. According to our results, central hypotensive effects may predominate over peripheral vasoconstrictive effects (Kuusela et al., 2001). Administration of alpha 2 adrenoceptor agonist caused reduction of cardiac output resulting from decrease in heart rate and contractility (Muir and Piper, 1977) leading to diminish in mean arterial pressure. However, the peripheral effects by the action of rilmenidine and moxonidine, as blockade of vascular alpha1-adrenoceptors and activation of presynaptic inhibitory alpha 2-adrenoceptors on axon endings of postganglionic sympathetic neurons contributing to the hypotension have been noted (Bock et al., 1999).

In the present study, yohimbine, an alpha 2-adrenoceptor antagonist completely reversed the effects of brimonidine on mean arterial pressure and heart rate and tended to be higher than baseline level. Bock et al. (1999) reported a similar manner after administration of the antagonists to rilmenidine- and moxonidine-treated rabbits. The blood pressure and plasma norepinephrine concentration rose to values higher than the initial ones. Possibly, this was an acute withdrawal phenomenon. An antagonist-elicited acute withdrawal response by increasing the activity of locus coeruleus neurons which can be observed 75 min after administration of clonidine (Duggan et al., 1994).

The value of MAP/R-R interval of ECG (rate-pressure product (HR*MAP)) has been used to estimate myocardial oxygen consumption (Pypendop and Verstegen, 2000). The rate pressure product decreased in response to brimonidine administration. Peak of effect was in the second-hour of duration of action in both dose groups. The decline in rate-pressure product following brimonidine administration, was the result of bradycardia coupled with systemic hypotension suggesting impaired baroreflex function. The medial nucleus of the solitary tract (NTS) in the caudal medulla is the primary central termination site for baroreceptor afferents. The integrity of this region

is necessary for normal baroreflex control of blood pressure (Hayward et al., 2002). Because selective blockade of alpha 2-adrenoreceptors in the medial NTS of normotensive subjects can increase resting blood pressure and attenuate baroreflex function (Kubo et al., 1990; Sved et al., 1992; Yamazaki and Ninomiya, 1993). Therefore, the stimulation of alpha 2-adrenoreceptors produced effects which mimicked the stretch of baroreceptor and so result in reduce in mean arterial pressure and heart rate.

The increase in standard deviation of heart rate (SDHR) and mean arterial pressure (SDMAP) more than priming level were shown first-hour after brimonidine and yohimbine administrations. Brimonidine and yohimbine resulted in apparent decrease and increase in blood pressure and heart rate respectively in particular period. The more changes in blood pressure and heart rate, the more deviation of SDMAP and SDHR.

With the sympathoinhibition effect of brimonidine, vagal tone may be overdriven. The stimulation of particular receptors found in different regions of the brain including the NTS, a major center for autonomic control increases vagal tone and decreases sympathetic activity producing bradycardia and hypotension (Cullen, 1996). The elevation of vagal tone coupled with the decline in sympathetic activity may lead to prolong P-R interval but not P wave duration, which causes by impairing of electrical propagation in AV junction. For this particular reasons, the Q-T interval but not QRS duration were also prolonged which refered to the slowing down the propagation of action potential especially repolarization in ventricular myocardium. The Q-T interval varies inversely with heart rate: the faster the heart rate, the shorter the Q-T interval (Tilley, 1992). Yohimbine was able to reverse the effect of brimonidine, although P-R and Q-T interval was slightly diminished when compared to baseline values. The acute withdrawal phenomenon may play the important role to explain these alterations.

Glomerular filtration rate (GFR) decreased in response to brimonidine administration. Low dose brimonidine (0.2 mg/kg) appeared to affect the GFR more than high dose (0.5 mg/kg). Both effective renal plasma flow and renal blood flow declined after administration of brimonidine. These results contribute to non

significant change in filtration fraction. In addition, renal vascular resistance seems to be increased in response to brimonidine.

It has been known that hypotension is capable to activate pressor area in medulla and drives sympathetic signal to counteract the lowering of blood pressure. Baroreceptors are the major role of these processes to speed up heart rate, raise cardiac contractility, increase total peripheral resistance and also increase in renal sympathetic nerve activity (Vander, 1995).

Because of centrally sympathoinhibitory effect of alpha 2-adrenoceptor agonist, mean arterial pressure and heart rate which contributes to cardiac output appears to be reduced. Thereby the body system cannot maintain homeostasis of blood pressure and cardiac output due to loss of sympathetic responses. Particularly, renal sympathetic nerve activity becomes attenuated (Janssen et al., 2001). With intact sympathetic activity, renal vascular resistance would increase following the constriction of efferent arterioles. Net filtration pressure remained constant or decreased slightly, not nearly as much as renal blood flow does, it produces an increase in filtration fraction. In case of brimonidine, the absence of sympathetic outflow results in limited response of both arterioles. Hence, the decline in mean arterial pressure can produce equally decline in both renal blood flow and glomerular filtration pressure, and therefore with minimal change in filtration fraction.

Regardless of central acting, brimonidine can activated peripheral postsynaptic alpha 2-adrenoceptor results in increased systemic vascular resistance. Strandhoy (1985) demonstrated the increase in renal vascular resistance in response to either alpha 1 or alpha 2-adrenergic agonists. In the present study, the direct effect of brimonidine on renal vasoconstriction might influence predominantly at the afferent arteriole or minimally affected on either the afferent or efferent arterioles, because of the reduction in both ERPF and GFR.

In the higher dose group, the effective renal plasma flow, renal blood flow and glomerular filtration rate were less affected than the lower dose group. Centrally mediated sympathoinhibition produced paradoxical responses in renal function among low and high dose group of brimonidine administration. As described above,

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cardiovascular effect of brimonidine seem to be dose-dependent manner. On the other hand, the renal functions were less affected by higher dose-brimonidine. These findings may be due to the stronger sympathoinhibitory effect of the higher dose which resulted in lower renal sympathetic nerve activity and thus, less constrictive response of particular afferent arteriole. In addition, Takahashi, Hisa, and Satoh (1984) found that the release of both renin and prostagrandin via alpha-agonist in anesthetized dogs could be inhibited by yohimbine. This finding may be the reason that the high-dose of alpha 2-agonist do produce less degree for decreases in ERPF, ERBF and GFR. In other words, the more activation of alpha 2-adrenoceptor, the more release of renal vasodilator PGE_2.

Brimonidine increased fraction excretion of sodium. However, osmolar clearance, free water clearance and urine production were unchanged significantly. Ota et al. (1990) studied the effects of intracerebroventricular administration of central alpha 2-adrenoceptor agonists guanabenz on the regulation of renal water and electrolytes handling through endocrine, renal hemodynamics. They reported that guanabenz, alpha 2-agonist was capable to decrease arginine vasopressin release in anesthetized dogs. As guanabenz, brimonidine may reduce antidiuretic hormone from pituitary gland and therefore promoted water diuresis. However, the diuresis was not apparent in the present study.

Subthreshold renal nerve stimulation in the rat kidney induces sodium and water retention through activation of alpha 1-adrenoceptors, as shown by others in the rabbit and dog (Smyth, Umemura, and Pettinger, 1985). Hence, presynaptic alpha 2 inhibition of norepinephrine release may result in reduction of alpha 1-adrenoceptor stimulation and thereby promotes natriuresis. Additionally, the direct effect of alpha 2-adrenergic agonist on sodium reabsorption of kidney remains controversial (Feraille and Doucet, 2001)

De Fronzo et al. (1975) first demonstrated that insulin exerts an antinatriuretic effect independently of the glycemic status in healthy human. A similar effect was later report in dog (De Fronzo, Goldberg, and Agus, 1976) and rat (Kirchner, 1988). These results indicate that insulin may directly control renal sodium handling. Because insulin was shown to increase fluid and sodium in *in vitro* microperfused

rabbit proximal convoluted tubule (Baum, 1987). The antinatriuretic effect of insulin is thought to originate in part in proximal tubules. In the present study, insulin level was thought to be reduced by the direct effect of postsynaptic alpha 2-adrenoceptor stimulation on pancreatic beta cell (Cullen, 1996). Accordingly, the decline in plasma insulin level may result in the reduction of antinatriuretic effect on proximal tubule and hence increase renal sodium excretion.

Fractional excretion of potassium and inorganic phosphate was also elevated in response to brimonidine administration. Whether proximal or distal tubule is the site of action of brimonidine needs further investigation.

Brimonidine reduced respiratory rate significantly. Similar to other alpha 2 adrenoceptor agonist, the respiratory depression effect of dexmedetomidine occurred in rabbit (Zornow, 1991). Arata et al. (1998) analysed the modulation of respiratory neurons by epinephrine or norepinephrine in a newborn rat with brainstem-spinal cord preparation. The direct effects of epinephrine on pre-inspiratory (Pre-I) neurons were examined in a synaptic blockade solution (low Ca). The activity of Pre-I neurons could be directly regulated by excitation via alpha1-receptors and inhibition via alpha2-receptors. Therefore, respitatory depression in animals treated with brimonidine in the present study may be in part mediated by alpha2-adrenocertor in pre-inspiratory neuron.

There was no significant change in rectal temperature in response to brimonidine administration. Noradrenoceptors in the hypothalamus are depressed by alpha 2-agonist in a dose-dependent manner to cause hypothermia, a slight decrease in rectal temperature was observed in dogs sedated with medetomidine (Cullen, 1996). However, the hypothermic effect in the present study was not apparent. The peripheral vasoconstriction may occur in sedated dogs. After yohimbine administration, the temperature was slightly elevated. It may cause by the overflow of sympathetic activity following the withdrawal effect of alpha 2-adrenoceptor agonist (Bock et al., 1999) In this period, the dogs were more excite, alert, and responsive. Enormous muscular activity probably enhanced the body temperature.

Values of hematocrit and total protein changed with the same direction in response to brimonidine and yohimbine administration. Alpha 2-adrenoceptor stimulation produced increase in systemic vascular resistance mediated through post-synaptic alpha 2-adrenoceptor in both artery and vein (Cullen, 1996). It is quite difficult to initiate red blood cell redistribution to somewhere in body circulation because most of blood vessels were constricted simultaneously. In addition, the direct effect of clonidine, classical alpha 2 agonist, on the spleen was studies. Ojiri et al. (1993) reported no alteration in splenic diameter in response to intrasplenic artery injection of clonidine. Thus the spleen was not likely to be red blood cell-reservior in particular situation.

Bernstein et al. (2003) studied the relationship of plasma volume and sympathetic tone in nulliparous women and concluded that plasma volume was related inversely to both an estimate of alpha-adrenergic activation and heart rate. These findings were consistent with an adaptive physiologic response that was aimed at the maintenance of blood pressure in the face of reduced plasma volume. Hemodilution would be the answer of the question that how the hematocrit and total protein were reduced. The decrease in blood pressure in response to brimonidine resulted in reduction in vascular hydrostatic pressure. Consequently, interstitial fluid then moved into vascular space and diluted red blood cell and total protein in such compartment.

Blood glucose level tended to increase in both dosages of brimonidine. Alpha 2-adrenergic agonist induced diabetic effect in many studies. Medetomidine produced the elevated blood glucose by mediated through post-synaptic alpha 2 adrenoceptor in pancreatic beta cells (Cullen, 1996). In rabbit, the increase in blood glucose and the inhibition of insulin secretion with infusion of brimonidine (UK 14304) alone were antagonized previously treated with the very selective alpha 2-adrenoceptor antagonist 2-methoxyidazoxan (Garcia-Barrado el al., 1998).

Plasma concentration of potassium and phophate was elevated after brimonidine administration particularly in high-dose group. Insulin does not only increased membrane permeability for glucose, but also increases the cellular uptake of amino acids, potassium ions and phosphate ions (Guyton and Hall, 1996). Alpha 2-

adrenoceptor agonists were assumes to inhibit insulin secretion from pancreatic beta cell, hence, cellular uptake of potassium and phosphate ions was declined and plasma concentration of both electrolytes was increased.

In conclusion, brimonidine lowered heart rate, blood pressure, respiratory rate, renal blood flow and glomerular filtration rate. It enhanced natriuresis and elevated blood glucose level. These effects can be reversed by specific alpha 2-adrenoceptor antagonist yohimbine hydrochloride.