# CHAPTER 2

## LITERATURE REVIEW

Brimonidine tartrate, 0.2% ophthalmic solution (Alphagan®) is a relatively selective alpha-2 adrenergic agonist. The chemical name of brimonidine tartrate is 5-bromo-6-(2-imidazolidinylideneamino) quinoxaline L-tartrate. It has a molecular weight of 442.24 as the tartrate salt and is water soluble (34mg/ml) with pH of 6.5. The molecular formula is $C_{11}H_{10}BrN_5 .C_4H_6O_6$. The chemical structure is shown in figure 2.1. Brimonidine is preserved in benzalkonium chloride (0.05 mg). It is structurally similar to clonidine and apraclonidine. It is more lipophilic and alpha-2 selective than apraclonidine (McGhie, 2001). A peak ocular hypotensive effect occurs at two hours post-dosing. After ocular administration of a 0.2% solution, plasma concentrations peaked within 1 to 4 hours and declined with a systemic half-life of approximately 3 hours (Allergan, 2001).

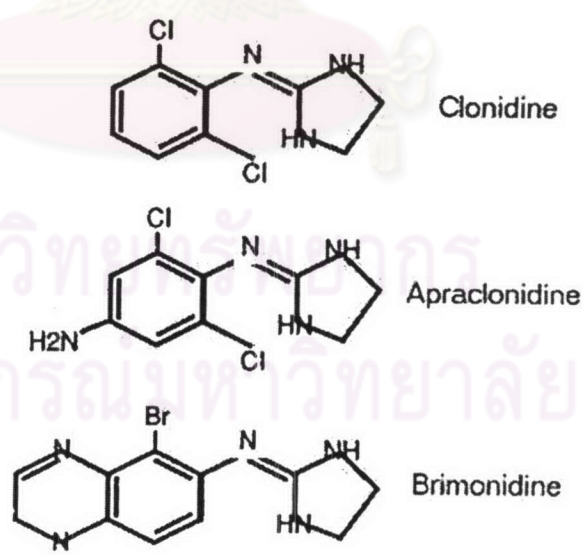


**Figure 2.1-** the chemical structure of brimonidine in compare to clonidine and apraclonidine.

Acheampong et al. (1996) studied *in vitro* metabolism of $^{14}$C-brimonidine in rat, rabbit, dog, monkey and human liver fractions. *In vitro* metabolism with rabbit liver aldehyde oxidase and human liver slices, and *in vivo* metabolism in rats were also investigated. The hepatic and urinary metabolites were characterized by liquid chromatography and mass spectrometry. Hepatic oxidation via hepatic aldehyde oxidase of brimonidine to 2-oxobrimonidine, 3-oxobrimonidine and 2,3-dioxobrimonidine was a major pathway in all species studied, except the dog whose prominent metabolites were 4',5'- dehydrobrimonidine and 5-bromo-6-guanidinoquinoxaline. The results indicated extensive hepatic metabolism of brimonidine and provided evidence for aldehyde oxidase involvement in brimonidine metabolism. There are species differences in hepatic brimonidine metabolism for example in dogs, which are likely related to the different in hepatic aldehyde oxidase activity.

## Clinical application of brimonidine

Glaucoma accounts for approximately 15% of blindness worldwide and is the leading cause of irreversible blindness. It is defined as an optic neuropathy that leads to optic nerve tissue loss and the cupping or excavation of the nerve head (McGhie, 2001). Gelatt (2000) stated that all glaucomas are diseases of changes, and the glaucomas consist of five stages:

1. An initial event or series of events,
2. An obstruction of the aqueous humor outflow system resulting from the initial event or series of events,
3. An increased intraocular pressure (IOP) that is too high for optic nerve axoplasmic flow and blood flow,
4. A retinal ganglion cell (RGC) dysfunction with resulting optic nerve degeneration and atrophy, and
5. Visual-field loss and blindness

Medical therapy is the first line of attack against primary open-angle glaucoma. Beta-blockers, miotics, sympathomimetics, carbonic anhydrase inhibitors and prostaglandins have been used with varying degree of success (Wilensky, 1996).

Since its introduction in 1996, use of brimonidine tartrate 0.2% ophthalmic solution (Alphagan®), a highly selective alpha 2-adrenergic agonist, has become increasingly popular for the initial and long-term management of ocular hypertension and glaucoma (Cantor, 2000).

Action of Brimonidine is relatively alpha-agonist, which reduces intraocular pressure (IOP) by decreasing aqueous production and increasing uveoscleral outflow (Greenfield et al., 1997; Enyedi and Freedman, 2001). Neuroprotective effect of brimonidine, which is an important feature in the new contexts of glaucoma pathogenesis, seems to potentiate the use of brimonidine in glaucoma and ocular hypertensive patient over other drugs. Neuronal degeneration can be grouped into 2 categories depending on the location, the primary insult-somogenic and axogenic (Gao et al., 2002). Lafuente et al. (2001) studied the dose-response effects of topically administered brimonidine on retinal ganglion cell survival, short and long periods of time after transient retinal ischemia. They found that topical pretreatment with brimonidine prevents ischemia-induced retinal ganglionic cell death in a dose-dependent manner.

Brain derived neurotrophic factor seems to play the important role in neuroprotection. In experimental studies, increased intraocular pressure induced by laser photocoagulation of episcleral and limbal veins was used to investigate the neuroprotective effect of brimonidine on eyes, by injecting brimonidine tartrate ophthalmic solution intravitreally. Control eyes were injected with balance salt solution. Brimonidine was sufficient to significantly increase endogenous brain derived neurotrophic factor (BDNF) expression in Retinal ganglion cells which determined by northern blot analysis, associated with increasing in number of BDNP-positive cell as dose dependent manner. Hence the neuroprotective effect of brimonidine may be mediated by the increase in BDNF expression (Gao et al., 2002).

The ocular hypotensive and side effects of brimonidine were determined for underlying mechanisms in conscious rabbits and cynomolgus monkeys. The objective was to determine the intraocular pressure (IOP) response to brimonidine in rabbits and monkeys and side effects (miosis, cardiovascular depression) in monkeys. Conscious albino rabbits and cynomolgus monkeys were pretreated topically with the following

receptor antagonists: rauwolscine (alpha-2), idazoxan (alpha-2 and imidazoline receptor), SKF 105854 (vascular postjunctional alpha-2), and prazosin (alpha-1). Intraocular pressure, pupil size and blood pressure/ heart rate were monitored noninvasively for 6 hours following dosing. Binding experiments were performed using [3H]brimonidine in membrane preparations from rabbit iris/ciliary body and from monkey cerebral cortex and brain stem. In rabbits, the ocular hypotensive response to brimonidine was unilateral and was inhibited by rauwolscine > idazoxan >> SKF 105854 = prazosin; this ranked order of potency correlated with displacement of [3H]brimonidine in the rabbit iris/ciliary body. In monkeys, brimonidine decreased IOP bilaterally and suppressed cardiovascular function suggesting a CNS site of action. Intraocular pressure and cardiovascular responses to brimonidine were inhibited by idazoxan >> rauwolscine > SKF 105854 = prazosin. A similar profile was obtained for displacement of [3H]brimonidine in monkey brain tissue. Both rauwolscine and idazoxan inhibited the miotic response to brimonidine in monkeys. Taken together, these results indicate that brimonidine stimulates an ocular alpha-2 adrenoceptor to decrease IOP in the rabbit and a CNS imidazoline receptor to decrease IOP, blood pressure, and heart rate in the cynomolgus monkey. The miotic response in the monkey is mediated by an alpha-2 adrenoceptor. The alpha-1 and vascular postjunctional alpha-2 adrenoceptors do not appear to play a role in mediating these responses (Burke et al., 1995).

In conscious cynomolgus monkeys, ocular hypotensive and side effects of brimonidine were determined. The result showed that brimonidine can decrease IOP bilaterally and suppressed cardiovascular function suggesting a CNS site of action. Another experiment was conducted in ketamine-anesthetized normal cynomolgus monkeys. Topical brimonidine produced a dose-dependent reduction in mean arterial blood pressure and heart rate (Gabelt et al., 1994).

There was only one case report mentioned about the toxicity of brimonidine in dogs. Welch and Richardson (2002) discovered that between January 1998 and December 2000, the ASPCA Animal Poison Control Center (APCC) received 52 calls concerning brimonidine ophthalmic solution ingestion in dogs. The report summarized typical clinical signs and characterized the anticipated course of

brimonidine toxicoses in dogs. Clinical signs developed within 2-4 hours. Incidence of clinical signs reported included bradycardia (67%), depression (46%), ataxia (27%), hypotension (25%), pallor (23%), weakness (17%), change in mucous membrane color (17%), hypothermia (13%), and vomiting or retching (13%). Other signs reported were shock, weak pulse and poor capillary refill time. Clinical signs seen following brimonidine ophthalmic drops ingestion in dogs are similar to systemic effects reported after ocular administration in infants, i.e. hypotension, bradycardia, and CNS depression.

Brimonidine has been evaluated in a number of safety studies using doses much greater than those in human. The results of the 6-month ocular/systemic study in rabbits and the 1-year ocular/systemic study in monkeys with 0.2, 0.5, and 0.8% brimonidine ophthalmic formulations showed no ocular or organ toxicity. The highest concentration of 0.8% used in rabbits and monkeys resulted in plasma drug concentrations of 95 (Cmax) and 10 (C2hr) times, respectively, higher than those seen in human following topical dosing. In the 1-year oral study of monkeys, transient exaggerated pharmacological effects of sedation were dose-related and no any organ toxicity was observed. The dose which elicited an apparent pharmacologic effect produced a plasma drug concentration approximately 115 times higher than in human. In 2-year carcinogenicity studies in mice and rats using doses that produced plasma concentrations 77 and 118 times, respectively, higher than those seen in human, no oncogenic effect was observed. Based on the extensive safety research on brimonidine, it was concluded that this drug has an excellent safety profile (Angelov et al., 1996).

Specific alpha-2 antagonist can reverse brimonidine toxicoses. Yohimbine (0.1mg/kg intravenously) or atipamezole (50μg/kg intramuscularly), have been used with consistent success in reversing hypotension and bradycardia. Brimonidine-induced respiratory depression, hypotension, and coma may respond to naloxone (0.02mg/kg iv or im) (Welch and Richardson, 2002). However, Sztajnbok (2002) described a 6-week-old girl in which a large naloxone dose failed to reverse effects induced by brimonidine.

## Molecular biological studies of alpha 2-adrenoceptor agonists

Many investigators were interested in molecular mechanisms of brimonidine in various species. Brimonidine is a selective and potent alpha 2-adrenoceptor agonist and also binds to nonadrenergic imidazoline receptors (Sigma, 1999). Brimonidine is 1,000 times more selective for alpha 2 than alpha 1-adrenoceptor (Welch and Richardson, 2002). It also exhibits agonist activity at 5-$HT_{1A}$ receptors *in vitro* (Newman-Tancredi et al., 1998).

Guyenet (1997) provided good review of whether hypotensive effect of clonidine and related drugs was due to imidazoline or alpha 2-binding sites. Clonidine and other related alpha 2-adrenoceptor agonists lower arterial pressure primarily by an action within the central nervous system. These drugs also have varying degrees of affinity for other cellular components called nonadrenergic imidazoline binding sites (NAIBS). For over 20 years, the alpha 2-adrenoceptor agonist activity of clonidine-like drugs was thought to account for their therapeutic effects (alpha $_2$-theory). However, several groups have recently proposed a competing "imidazoline theory". Accordingly, the hypotensive effect of clonidine-like drugs would be in fact responsible more to their affinity for one type of NAIBS, called $I_1$ receptors.

The alpha 2-theory is strongly supported by several findings. inFirst, the hypotensive effect of systemically administered clonidine is blocked by alpha 2-adrenoceptor antagonists that are without affinity for $I_1$ NAIBs. Second, the hypotensive effect of intravenous clonidine is absent in genetically engineered mice in which a defective alpha 2A-adrenoceptor has been substituted for the normal one. Third, the sympatholytic effect of clonidine is consistent with the presence of conventional inhibitory alpha 2-adrenoceptors on sympathetic preganglionic neurons and on their main excitatory inputs in the medulla oblongata. Fourth, the first $I_1$ ligand without affinity for alpha 2-adrenoceptors was found to be biologically inactive. The imidazoline theory is supported by a limited data of whole animal "*in vivo*" pharmacological experiments that remain open to a wide range of interpretations. For these reasons, many evidences strongly supported a more predominant role of alpha 2-adrenoceptor mechanisms in the action of most clonidine-like agents in compare

with nonadrenergic imidazoline binding sites at therapeutically relevant doses or concentrations (Guyenet, 1997).

Activation of alpha 2-adrenoreceptors can result in the regulation of multiple signaling pathways and protein targets. Some of which are thought to be potentially neuroprotective. Alpha 2-adrenoceptor agonists via its interaction with the pertussis toxin-sensitive Gi/G(o) class of G proteins, modulates multiple effector systems including inhibition of adenylyl cyclase, voltage gated $Ca^{2+}$channels and activation of inward rectifying $K^+$ channels. These changes hyperpolarize neurons and inhibit presynaptic neurotransmitter release (Lakhlani et al., 1996). Alpha 2-adrenoceptor agonists also induced the phosphorylation of mitogen-activated protein kinase and inhibited the cAMP-dependent phosphorylation of its response element binding protein (Alblas et al., 1993; Fitzgerald et al., 1999).

Functional antagonism of alpha 2-adrenoceptors on the induction of cyclic adenosine monophosphate (cAMP) by vasopressin was demonstrated in rat but not in the dog or cynomolgus monkey. The effect of the novel alpha-2 adrenoceptor agonist, AGN 190851, was evaluated for its diuretic action in the rat, dog and cynomolgus monkey. Its ability to inhibit vasopressin-stimulated cAMP accumulation in rat and dog cortical collecting tubules *in vitro* was also demonstrated. The data indicate that in the rat, AGN 190851 resulted in a dose-dependent water diuresis, which was accompanied by an increase in blood pressure and osmolar clearance. In addition, AGN 190851 resulted in a dose-dependent inhibition of vasopressin-stimulated cAMP accumulation in rat cortical collecting tubules *in vitro*. In contrast, AGN 190851 was unable to cause either a water diuresis in conscious dogs or inhibit vasopressin-stimulated adenylate cyclase activity in canine tissue *in vitro*. In anesthetized cynomolgus monkey, AGN 190851 also failed to alter renal function significantly. When administered the vasopressin receptor antagonist, SK&F 105494 to either dogs or cynomolgus monkeys, the antagonism of the vasopressin V2 receptor which results in a brisk water diuresis was observed in both species (Brooks et al., 1991). Thereby the alpha 2-adrenoceptor agonist induced water diuresis was more prominent in rat than dog or cynomolgus monkey.

In rat nephrons, alpha 2-adrenoceptors activation could inhibit cAMP formation stimulated by parathormone in proximal convoluted tubules and by arginine vasopressin in cortical and medullay collecting tubules but not cortical and medullary thick ascending limb of Henle's loops. Umemura et al. (1985) examined the effect of alpha 2-adrenoceptor stimulation with epinephrine (E) on cell cAMP content in the isolated rat proximal convoluted tubule (PCT), medullary and cortical thick ascending limb of Henle and collecting tubule (MTAL, CTAL, MCT, and CCT) respectively. Parathyroid hormone (1-34 PTH) in PCT or CTAL or arginine vasopressin (AVP), in MTAL, CTAL, MCT, or CCT, was used to activate adenylate cyclase in microdissected nephron segments in the presence of 3-isobutyl-1-methylxanthine (phosphodiesterase inhibitor) and propranolol. Alpha 2-adrenoceptors were activated using varying concentrations of E (37°C, 2 min of duration). Alpha 2-adrenoceptor activation with E ($5 \times 10^{(-7)}$ to $5 \times 10^{(-6)}$ M) suppressed cellular cAMP stimulation by PTH 35% in PCT and by AVP 50% in CCT. This suppression by E in PCT and CCT was inhibited by $5 \times 10^{(-6)}$ M yohimbine or $5 \times 10^{(-7)}$ M phentolamine but not by $5 \times 10^{(-6)}$ M prazosin. Epinephrine also suppressed cAMP stimulated by AVP in MCT but it did not suppress the PTH-or AVP-stimulated increase in cellular cAMP in CTAL and MTAL. These studies showed that there are alpha 2-adrenoceptors in the rat nephron. Activation of these alpha 2-adrenoceptors can inhibit cAMP formation stimulated by PTH in PCT and by AVP in the CCT and MCT but not in the CTAL and MTAL.

There was an evidence of the increase in inositol-1,4,5-trisphosphate formation by 4 to 6-fold over control and diacylglycerol formation by 46% in response to alpha 2-adrenoceptor agonist B-HT 933 in distal convoluted tubular (DCT) cells. Basal intracellular calcium concentration in single DCT cells averaged 114 nM and increased within 2 min to 196 nM with B-HT 933 and could be antagonized by specific phospholipase C antagonist U-73122 but not pertussis toxin. These findings provide evidence that alpha 2-adrenoceptors activate phospholipase C in DCT cells through a pertussis toxin-insensitive mechanism (Gesek, 1996).

## Distribution of alpha 2-adrenoceptor in the body system

Central alpha 2-adrenoceptors are present in brainstem neurons that control sympathetic tone. In particular, immunoreactivity for alpha2 adrenoceptors are present in most bulbospinal cells that contribute an excitatory input to vasomotor sympathetic preganglionic neurons, including the C1 and A5 catecholaminergic neurons of the ventrolateral medulla and the serotonergic cells of the medullary raphe (Guyenet, 1997).

Alpha 2-adrenoceptors were also found in many tissues in cardiovascular systems. Canine venous system study showed the distribution of alpha 2-adrenoceptor in many veins but higher response of clonidine were significant found in the saphenous, cephalic, femoral and external jugular veins. These receptor sites were also present in mesenteric and portal vein, and inferior vena cava (Shoji, Tsuru and Shigei, 1983). Alpha 2-adrenoceptors were also found in many arteries. Coronary blood flow velocity and coronary flow reserve can be modulated by this particular activated receptor and alpha -adrenergic microvascular coronary constriction is predominantly mediated by $alpha_2$-adrenoceptors in dogs and in humans (Gregorini et al., 2002). In human, Chotani et al. (2004) demonstrated site-specific expression of alpha2-adrenoceptors in human vascular smooth muscles that reflected differential activity of alpha 2-adrenoceptor gene promoters. The high expression was found in venous and arteriolar vascular smooth muscles without detectable expression or function in aortic vascular smooth muscles.

Many studies reported alpha 2 –adrenoceptor action in kidneys. Evans and Anderson (1995) studied the renal effects of the alpha 2-adrenoceptor agonists, rilmenidine and guanabenz and the antagonists, 2-methoxyidazoxan and idazoxan, in conscious dogs. Since the effects of these drugs on renal function could be mediated in the central nervous system or periphery, the dogs were studied under both normal and ganglion-blocked conditions. In dogs with intact autonomic reflexes, 2-methoxyidazoxan (15 micrograms kg-1 plus 0.6 micrograms kg-1 min-1) produced effects consistent with a generalized increase in sympathetic drive, including increases in mean arterial pressure and plasma renin activity, and a reduction in

sodium excretion. In ganglion-blocked dogs, 2-methoxyidazoxan reduced sodium excretion but had no discernible effect on systemic or renal haemodynamics. Therefore, intra renal alpha 2-adrenoceptor may be the important role in renal sodium excretion. At least three isoforms of alpha 2-adrenoceptors (alpha $_{2A}$, alpha $_{2B}$, alpha $_{2C}$) account for the pharmacologically defined alpha 2- adrenoceptors. These receptor are present in various sites of kidney both in cortex and medulla (Feraille and Doucet, 2001), most experiments were performed in rats and rabbits while alpha 2 adrenoceptors in dog kidney remain to be addressed.

## Cardiovascular effects of alpha 2-adrenoceptor agonists

Alpha 2-adrenoceptor agonists produced changes to cardiovascular system, mostly through stimulation of central receptors (Cullen, 1996). However, peripheral receptors can be activated by these particular agonists. Stimulation of receptors found in different regions of the brain including the nucleus tractus solitarius, a major center for autonomic control, by increased vagal tone and decreased sympathetic activity producing bradycardia and hypotension. Pharmacological studies using receptor selective agonists and antagonists have shown peripheral alpha 2-adrenoceptors to be located in either the presynaptic or postsynaptic position. Presynaptic receptor stimulation inhibits norepinephrine release further, reducing sympathetic tone and contributing to bradycardia. Stimulation of postsynaptic receptors in the walls of arteries and veins has a vasopressor effect (Cullen, 1996).

The central effect of alpha 2 –adrenoceptor agonists is general agreement that a major site of action of these drugs is the rostral ventrolateral medulla (RVLM), but their precise site of action within this structure remains unknown. The RVLM harbors bulbospinal neurons that send a monosynaptic excitatory projection (presynaptic neurons) to sympathetic preganglionic neurons. These neurons are active *in vivo* and receive convergent excitatory and some of these cells are inhibited by iontophoretic application of alpha 2-adrenoceptor agonists *in vivo*. Postsynaptic inhibition of RVLM presympathetic neurons probably contributes to the sympatholytic action of these substances (Allen and Guyenet, 1993). In support of this interpretation, alpha 2-adrenoceptor agonists activate an inwardly rectifying potassium current in many

RVLM bulbospinal neurons *in vitro* (Li, Bayliss and Guyenet, 1995), and the presynaptic neurons that have a catecholaminergic phenotype (C1 cells) express alpha 2A-adrenoceptors as known in immunocytochemical studies (Guyenet et al., 1994). Hayar and Guyenet (1999) proposed a hypothetical model explained presynaptic and postsynaptic alpha 2-adrenoceptor inhibition of presympathetic neuron which exhibited sympathoinhibitory effect as shown in figure 2.2.

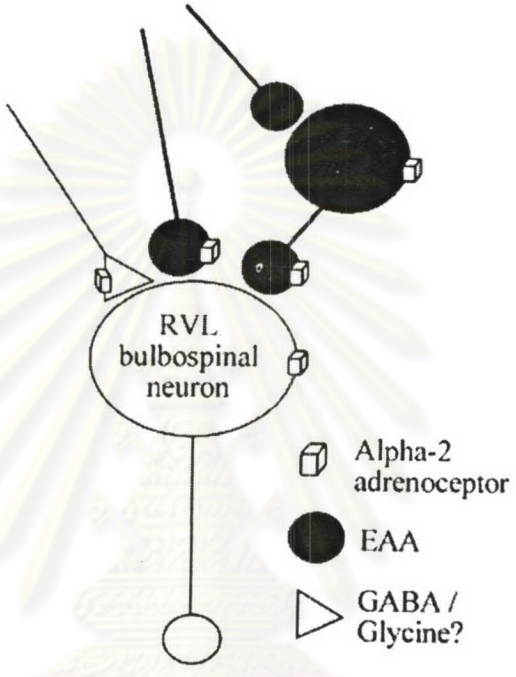


**Figure 2.2**- Presumed location of presynaptic and postsynaptic alpha 2-adrenoceptors in RVLM. About 70% of RVLM bulbospinal neurons have postsynaptic alpha 2-adrenoceptors that are coupled to a potassium conductance. Input to these cells is predominantly excitatory and originates in part from local excitatory interneurons. Norepinephrine inhibits release of excitatory amino acids (EAA) by activating alpha 2-adrenoceptors located on presynaptic terminals and possibly also on soma of local excitatory neurons. Finally, RVLM bulbospinal neurons receive an inhibitory input that is predominantly GABAergic and subject to inhibition by alpha 2-adrenoceptor agonists.

There was some evidences indicated that peripheral alpha 2-adrenergic effect are capable to elicit vasoconstriction. Romifidine, another alpha 2-adrenoceptor agonist can activate vascular smooth muscle-alpha 2-adrenoceptors and produced vasoconstriction. Pypendop and Verstegen (2000) studied the romifidine effects on cardiovascular system in dogs. Romifidine was administered intravenously at a dose

of 5, 10, 25, 50 and 100 μg/kg into 5 groups of dog. Heart rate, pulmonary arterial pressure, rate-pressure product, cardiac index, and right ventricular stroke work index were decreased while central venous pressure, pulmonary capillary wedge pressure and systemic vascular resistance index were increased in response to romifidine administration. The increased systemic vascular resistance before the decline of heart rate and blood pressure may contributed to the initial hypertension observed in this experiment.

In addition, alpha 2-adrenoceptor agonists may produce change in plasma volume. Bernstein et al. (2003) studied the relationship of plasma volume to sympathetic tone in nulliparous women and concluded that plasma volume was related inversely to both an estimate of alpha-adrenergic activation and heart rate. Hematocrit and concentration of other blood compositions may be altered in response to alpha 2 adrenoceptor agonists.

## Renal effects of alpha 2-adrenoceptor agonists

As we known, alpha 2-adrenoceptors were present in many regions of kidney. In rat and human proximal tubules, alpha 2-adrenoceptors are twice more abundant than alpha1-adrenoceptors and are mainly accounted for by the $alpha_{2B}$-isoform present at the basolateral border. In contrast, the alpha 2A/C receptor isoform accounts for alpha 2-adrenoceptors expressed in outer medullary collecting duct where alpha $2_B$ is undetectable (Ferialle and Doucet, 2001).

Menegaz, Kapusta, and Cabral (2000) studied the role of intrarenal alpha 2-adrenoceptors in the renal responses to xylazine in rats. They examined the contribution of intrarenal alpha 2-adrenoceptor mechanisms to enhance urine flow rate (V) and urinary sodium excretion ($U_{Na}V$) in ketamine-xylazine-anesthetized rats. They demonstrated that injection of 5 μg of yohimbine into left renal artery significantly reduced urine flow rate and urinary sodium excretion in experimental (left) but not in the control (right) kidney. In related studies, Blandford and Smyth (1988) demonstrated that the intrarenal infusion of clonidine produced a dose related

increase in urine flow rate. In their studies, however, an increase in urinary sodium excretion and osmolar clearance were only observed at the highest infusion rate.

Norepinephrine activated alpha 1-adrenoceptor can inhibit the excretion of sodium and water. The stimulatory effect of norepinephrine on proximal tubule reabsorption is mediated at least in part by alpha 1-adrenoceptors. Prazosin, specific alpha 1-antagonist, decrease chloride reabsorption in the in situ microperfused rat proximal convoluted tubule and antagonized in bicarbonate reabsorption induced by renal nerve stimulation in anesthetized dogs (Feraille and Doucet, 2001). The mechanism of alpha 2 mediated the renal excretion of sodium and water may involve the detrimental effect of renal sympathetic nerve activity which mediated through alpha 1-adrenoceptor. Menegaz et al. (2000) proposed that in intact rats, an intrarenal component of the diuresis and natriuresis produced by intravenous xylazine infusion was mediated by the stimulation of presynaptic alpha 2-adrenoceptors located on renal nerve terminals since the renal effects was completely abolished by yohimbine. Activation of presynaptic alpha 2-adrenoceptors on renal sympathetic nerve terminals would inhibit the neural release of norepinephrine and promote water and sodium excretion that can be reversed by yohimbine.

The site of increase urinary sodium and water excretion observed in response to an alpha 2-adrenoceptor agonist was localized in the collecting duct by micropuncture studies in rat (Stanton, Puglisi and Gellai, 1987). Conversely, clonidine altered neither water permeability nor sodium reabsorption promoted by vasopressin in the cortical collecting ducts of rabbits (Chen, Reif and Schafer, 1991) dogs and cynomolgus monkeys (Brooks et al., 1991). Hence alpha 2-adrenoceptor agonists mediated vasopressin action was species specific. Moreover, alpha 2-adrenoceptor agonists can stimulate postsynaptic alpha 2-adrenoceptor and resulted in the increase of renal vascular resistance in dogs while have almost no effect on renal circulation in rat (Strandhoy, 1985).

In addition, central effect of alpha 2-adrenergic agonists on vasopressin release was also mentioned. Alpha 2-adernoceptor agonist guanabenz injected intracerebroventriculary can produce the reduction in aldosterone and arginine vasopressin release in anesthetized dogs (Ota et al., 1990). On the other hand, the

effect of alpha 2-adrenoceptor agonists on renal blood flow and glomerular filtration rate may mediate through central sympathoinhibitory effect which influenced systemic hemodynamic regulation reported in many studies (Cullen, 1996; Hayar and Guyenet, 1999; Hayar and Guyenet, 2000). In pithed rat which excluded renal sympathetic nerve activity, alpha2-adrenoceptor agonist was capable to increase and then decrease in renal blood flow (Richer et al., 1987) which response to biphasic effect on blood pressure.

## Effects of alpha 2-adrenoceptor agonists on other organ systems

Alpha 2-adrenoceptor agonists affected the glucose metabolism via interfering the insulin release. In conscious fasted rabbits, the insulin secretory response induced by the intravenous infusion of the alpha1-adrenoceptor agonist, amidephrine was blocked by the simultaneous administration of clonidine. The excitatory effect of amidephrine on insulin secretion was similarly suppressed by the concomitant infusion of the selective alpha 2-adrenoceptor agonist UK14304 (brimonidine) (Garcia-Barrado et al., 1998). In transgenic mice which overexpressed alpha2 adrenoceptors in pancreatic beta-cells showed altered regulation of glucose homeostasis. They had normal glycemia and insulinaemia in basal conditions but greater hyperglycemic and hypoinsulinaemic responses after injection of the alpha 2-agonist, UK14304. The lower blood insulin concentration detected in transgenic mice was a reflection a stronger inhibitory effect of the alpha2-agonist on glucose-stimulated insulin secretion in transgenic islets than in controls (Devedjian et al., 2000).

Respiratory depression may be one of the consequences on alpha 2-agonist administration. Arata, Onimaru, and Homma (1998) analysed the modulation of respiratory neurons by epinephrine or norepinephrine in a newborn rat brainstem-spinal cord preparation. The direct effects of epinephrine on pre-inspiratory (Pre-I) neurons were examined in a synaptic blockade solution (low Ca), and fifty-six percent of Pre-I neurons were found to continue firing. In low-Ca solution, Pre-I neurons were excited (n=29 of 39) or depressed (n=5 of 39) by epinephrine, and excited by alpha1-agonist phenylephrine or depressed by alpha 2-agonist clonidine. These finding

indicated that the activity of Pre-I neurons could be directly excitated via alpha1-receptors and inhibited via alpha 2-receptors.