

# The $\alpha_2$ -adrenergic receptors in hypertension and heart failure: experimental and clinical studies

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This is a brief overview of experimental and clinical studies exploring the hemodynamic functions of the  $\alpha_{2A}$  and  $\alpha_{2B}$  adrenergic receptor (AR) subtypes in animals submitted to genetic manipulations or gene treatment, as well as the clinical effects of central sympathetic suppression with the  $\alpha_2$ -AR agonist clonidine in patients with ischemic heart disease and/or heart failure. The animal experiments have led us to conclude that the sympathetic outflow is regulated by activation of the presynaptic  $\alpha_{2A}$ -AR subtype, which is the predominant  $\alpha_2$ -AR subtype in the central nervous system and exerts a sympathoinhibitory (hypotensive) action; on the contrary, activation of the central  $\alpha_{2B}$ -AR elicits a sympathoexcitatory response (such as seen in salt-induced hypertension, which requires functionally intact  $\alpha_{2B}$ -AR). Since there are no selective pharmacologic agents yet capable of discriminating among  $\alpha_2$ -AR subtypes, clinical studies utilize clonidine, the central sympathetic suppressant effect of which has been used for 35 years to treat hypertension. In small clinical trials, clonidine was used successfully for treatment of acute or chronic heart failure, acute myocardial infarct or

hypertensive cardiomyopathy with subclinical diastolic dysfunction. We speculate that future development of agents capable of selectively activating the  $\alpha_{2A}$ -AR or blocking the  $\alpha_{2B}$ -AR may further improve our capability to treat hypertension, ischemic heart disease and heart failure. *J Hypertens* 19:2115–2124 © 2001 Lippincott Williams & Wilkins.

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## Introduction

The sympathetic nervous system (SNS) plays a pivotal role in the regulation of blood pressure and cardiac function. The effects of catecholamines and sympathomimetic agents, acting as both hormones and neurotransmitters, are mediated via adrenergic receptors and dopaminergic receptors. There are three types of adrenergic receptors (ARs), with three subtypes cloned for each one:  $\alpha_{1A}$ ,  $\alpha_{1B}$ ,  $\alpha_{1D}$ ,  $\alpha_{2A/D}$ ,  $\alpha_{2B}$ ,  $\alpha_{2C}$ ,  $\beta_1$ ,  $\beta_2$ ,  $\beta_3$  [1]; and five subtypes of the dopaminergic receptor.

The  $\alpha_2$ -AR has long been known to regulate the sympathetic outflow from the central nervous system (CNS) and the release of norepinephrine. Indeed, the  $\alpha_2$ -AR agonist clonidine was introduced as an antihypertensive SNS suppressant 35 years ago and continues to be the main pharmacologic agent of its class. This article will briefly review recent advances that have linked the  $\alpha_2$ -AR to salt-induced hypertension and have used genetic engineering and gene treatment to elucidate the function of  $\alpha_2$ -AR subtypes, of which a more extensive account was recently published elsewhere [2]; it will also summarize clinical studies that have explored the application of central SNS suppression to the treatment of ischemic heart disease and heart failure.

## How does salt raise blood pressure?

A large body of literature has established the crucial role of the kidney in retaining salt and fluid, thus causing hypertension. This led to the mechanistic concept of 'volume expanded' hypertension, which implied that retention of salt and fluid would expand the intravascular volume space and increase hydrostatic pressure against the distended arterial walls. Yet, hypertension is characterized by increased systemic arterial resistance with decreased arterial lumen (through arterial wall constriction or structural remodeling) and does not require intravascular fluid volume expansion. In fact, true expansion of intravascular volume *per se*, (such as hemodilution in the syndrome of inappropriate antidiuretic hormone [SIADH] release) does not cause hypertension, and neither does retention of salt and fluid in the extracellular spaces, such as in various edematous states. We therefore set out to separate the role of fluid retention by the kidney from the hypertensive effect of salt [3].

## Experimental studies in rats

In order to bypass confounding effects of changes in renal excretory function, we first conducted a series of acute experiments, where anephric rats were infused

with isovolumic (2 ml) equiosmotic solutions of different ionic content. Infusion of 4% hypertonic saline produced BP increases by 27–30 mmHg, accompanied by sharp elevation of vasopressin and norepinephrine, causing intense vasoconstriction with contracted intravascular fluid volume [4–6]. Infusion of a 2 ml solution of 25% mannitol, which remained in the vascular space, produced a minimal BP rise with no neurohormonal activation. Further experiments in the late, salt-dependent phase of one-kidney, one-clip renovascular hypertension [7] and hypertension caused by subtotal nephrectomy and chronic renal failure [8] confirmed that the predominant hypertensive mechanism was central SNS stimulation. In other experiments, minute amounts of saline introduced directly in the CNS (via stereotaxically guided microinjection into the cerebral ventricles [9–11], or in selected areas of the brainstem, such as the nucleus tractus solitarius (NTS), locus coeruleus and ventrolateral medulla [12,13]) produced sharp increases in BP, accompanied by evidence of sympathetic stimulation and an increase in circulating catecholamine levels. By contrast, equiosmotic isovolumic solutions of dextrose or LiCl produced no such effect, suggesting that this was an ionic effect specific to NaCl.

Earlier studies by several investigators had demonstrated that destructive lesions in certain areas of the brainstem, including the NTS and the ventrolateral medulla, produced acute severe hypertension [14–17]. This severe hypertensive reaction was characterized by sympathetic overdrive, attributed to destruction of sympathoinhibitory neurons.

Evidence of sympathetic overactivity has also been established in various models of experimental salt-induced hypertension, where salt sensitivity may be either genetically determined [as in various strains of spontaneously hypertensive rats (SHR)] or acquired after ablation of renal mass with or without mineralocorticoid excess [7,8,18–22]. Actually, many of these and other studies also demonstrated a catalytic role of vasopressin, acting as a neurotransmitter/neuromodulator enhancing the responses of central adrenergic neurons [3,23–26]. Taken together, these studies would suggest that sodium loading leads to an increase in blood pressure associated with a hyperadrenergic state, which, in its earliest stages, is enhanced by the influence of vasopressinergic neurons; in the later stages of established salt-dependent hypertension, the prevalent mechanism seems to be sympathoexcitation resulting from disinhibition of adrenergic neurons or receptors located in the hypothalamus and brainstem.

Further support for this notion has come from experiments demonstrating that salt loading was associated with diminished concentration of catecholamines in

certain hypothalamic and brainstem areas of SHR [27–30], as well as alterations in the densities or ratios of  $\alpha_2$ -AR types [31–33] and altered affinity of  $\alpha_2$ -AR for agonists [34,35]. We therefore proposed the hypothesis that sodium decreases the affinity of central presynaptic sympathoinhibitory  $\alpha_2$ -ARs for naturally occurring agonists, thus resulting in sympathetic disinhibition and a hyperadrenergic state with elevated systemic blood pressure.

### Expression of $\alpha_2$ -AR genes in brain and arterial wall tissues

Since all of the above-cited studies suggested a crucial role for the  $\alpha_2$ -AR in salt-induced sympathetic activation, we sought to further define and localize the distribution of  $\alpha_2$ -AR subtypes in various tissues, especially in brain regions relevant to blood pressure regulation and in vascular wall tissues involved in vasoconstriction. The cloning of  $\alpha_2$ -AR subtypes made available cDNA probes that distinguish among subtype transcripts in RNase protection assays, thus permitting assessment of subtype-specific mRNA levels in selected tissue extracts. *In situ* hybridization followed by densitometry has allowed the comparative mapping of subtype expression in brain sections from selected areas of normotensive and hypertensive rats. Findings from such studies indicated that, after salt feeding, there were significant differences in the expression of  $\alpha_{2A}$ -AR versus  $\alpha_{2B}$ -AR subtypes in various areas of the cortex, brainstem and cerebellum between SHR and normotensive Wistar-Kyoto rats [36–39]. Although the functional significance of these differences is unclear, the very existence of such differences points further to their relevance in the sympathetic response to salt loading.

Notably, in arterial wall tissues from normal and atherosclerotic animals, we detected the presence of  $\alpha_{2A}$ -AR mRNA, but no  $\alpha_{2B}$ -AR mRNA [40]. This finding is in agreement with results reported by other investigators [41] and indicates that the  $\alpha_{2B}$ -AR subtype is not expressed in arterial wall structures.

### Deletion of $\alpha_2$ -AR gene subtypes in experimental animals

Because pharmacologic agents and radioligands lack the selectivity to differentiate between  $\alpha_2$ -AR subtypes, further definition of the functional characteristics of these subtypes requires genetic engineering. Genetic manipulation of mice has been used extensively in the past in order to explore the pathophysiologic role of proteins produced by the targeted genes. In order to evaluate the role of each  $\alpha_2$ -AR subtype in the induction and maintenance of hypertension, we conducted a series of experiments in genetically engineered mice, in which either the  $\alpha_{2A}$ -AR, the  $\alpha_{2B}$ -AR, or the  $\alpha_{2C}$ -AR gene was knocked out [42–46]. When subtotally ne-

phrectomized mice were submitted to chronic dietary salt loading, those deficient in  $\alpha_{2B}$ -AR had a greatly attenuated BP response, which never did reach hypertensive level, whereas those lacking the  $\alpha_{2A}$ -AR or  $\alpha_{2C}$ -AR subtype gene developed hypertension to the same extent as their wild-type counterparts [47,48]. Interestingly, the  $\alpha_{2A}$ -AR knockout mice, in which the  $\alpha_{2B}$ -AR acted unopposed, had already higher BP and circulating catecholamines at baseline and became hypertensive in less than 2 weeks, as compared to 4 or 5 weeks of salt loading required by the  $\alpha_{2C}$ -AR knockouts and all wild-type mice. These findings are consistent with those of other investigators who have suggested that central sympathetic outflow is regulated predominantly via the sympathoinhibitory presynaptic  $\alpha_{2A}$ -AR subtype, which exerts a hypotensive action [43–46]. The  $\alpha_{2C}$ -AR subtype seems to have no role in blood pressure regulation; indeed, most investigators believe that the  $\alpha_{2C}$ -AR influences mainly behavioral characteristics [49].

The major new finding from these studies was that  $\alpha_{2B}$ -AR-deficient mice are unable to develop salt-induced hypertension. Several mechanisms could account for this, for example: (1) abnormal renal handling of sodium (i.e. inability to retain sodium); (2) diminished capacity to release norepinephrine by central sympathetic neurons; (3) inability to vasoconstrict in response to circulating norepinephrine. Some investigators had suggested that the  $\alpha_{2B}$ -AR was exclusively located in the vascular wall, where its effect would be direct vasoconstriction [43,45]. However, as mentioned earlier, we have detected a high density of  $\alpha_{2B}$ -AR mRNA in areas of the brainstem [39] but not in the arterial wall [40], and other investigators have also been unable to detect expression of  $\alpha_{2B}$ -AR in vascular wall tissues [41]. This, along with the fact that catecholamine-induced vasoconstriction is mainly effected through the peripheral postsynaptic  $\alpha_1$ -AR [50], suggests that lack of vasoconstricting capacity is not a likely explanation.

Inability to retain sodium could be eliminated as a potential explanation by the use of anephric mice treated with hypertonic 4% saline infusion, so that accumulation of salt and fluid in the extracellular space would be equal in all animals. We used six groups of anephric mice, i.e. three groups of mice deficient for each one of the  $\alpha_2$ -AR subtypes, each one compared to its wild-type counterparts [51]. As expected, the  $\alpha_{2A}$ -AR gene knockouts started from a higher BP baseline, but all five subgroups (i.e. the  $\alpha_{2A}$ -AR knockouts, the  $\alpha_{2C}$ -AR knockouts and their respective wild-type controls, as well as the wild-type counterpart of the  $\alpha_{2B}$ -AR-deficient mice) developed similar acute BP elevations, ranging from 12–18 mmHg at the end of the 2 h hypertonic saline infusion; on the contrary, the  $\alpha_{2B}$ -AR-

deficient subgroup had an average 3 mmHg *decrease* of BP at the end of that period. All animals exhibited stimulated norepinephrine levels at the end of the infusion, and in all cases the  $\alpha_2$ -AR gene-deficient subgroups tended to have higher levels than their wild-type counterparts. However, the  $\alpha_{2B}$ -AR-deficient subgroup was the only one with significantly higher levels of norepinephrine after salt loading, thus eliminating the third explanation cited above. Actually, this surprising dissociation between heightened stimulation of norepinephrine release and decreasing levels of systemic BP was unexpected and remains difficult to explain.

Taken together with our earlier findings of increased density of  $\alpha_{2B}$ -AR mRNA in certain areas of the brainstem that represent centers of baroreflex control (such as the NTS and locus coeruleus [39]) and inability to detect  $\alpha_{2B}$ -AR mRNA in arterial walls [40,41], these data suggest that the  $\alpha_{2B}$ -AR has a hypertensive function mediated through the central SNS. On the basis of these findings, we have formulated the following hypothesis. Activation of the sympathoexcitatory  $\alpha_{2B}$ -AR would oppose the hypotensive sympathoinhibitory effect of the  $\alpha_{2A}$ -AR in the central SNS centers of vascular tone regulation. In such a case, excessive levels of circulating norepinephrine in  $\alpha_{2B}$ -AR-deficient mice would result in unopposed stimulation of the central presynaptic  $\alpha_{2A}$ -AR and therefore tend to further lower the systemic BP, which is precisely what we found in these mice. By contrast, in the  $\alpha_{2A}$ -AR gene knockout mice, unopposed function of central  $\alpha_{2B}$ -AR would lead to a hypertensive hyperadrenergic state, even at baseline; this state would be further exacerbated by the stimulus of salt-loading, as indeed was seen in the experiments with  $\alpha_{2A}$ -AR gene knockouts.

In order to demonstrate whether  $\alpha_{2B}$ -AR-deficient mice have the ability to vasoconstrict in direct response to catecholamines, we infused  $\alpha_2$ -AR agonists or antagonists in various combinations in mice deficient for each one of the  $\alpha_2$ -AR subtypes [52]. In summary, after blockade of the  $\alpha_1$ -AR with prazosin, a norepinephrine bolus produced a similar moderate pressor response in all wild-type as well as the  $\alpha_{2B}$ -AR- and  $\alpha_{2C}$ -AR-deficient mice, presumably via stimulation of one or both of the remainder  $\alpha_2$ -AR subtypes; however, in the  $\alpha_{2A}$ -AR knockouts, the same norepinephrine bolus produced a significant fall of BP by about 20 mmHg. This would indicate that the  $\alpha_{2A}$ -AR is responsible for the vascular wall sympathetic vasoconstrictive response in the absence of functional  $\alpha_1$ -AR. Indeed, when both  $\alpha_1$ -AR and  $\alpha_2$ -AR are blocked by concurrent administration of prazosin and yohimbine, the vascular response to norepinephrine bolus in all animals is relaxation (probably via  $\beta_2$ -AR stimulation). This re-

sponse indicates that the  $\alpha_{2A}$ -AR is the only vascular wall  $\alpha_2$ -AR subtype capable of direct vasoconstriction.

### Gene treatment via antisense oligonucleotides

Gene treatment has been used extensively in recent years for elucidation of the role of various proteins [53]. Delivery of antisense oligodeoxynucleotides (AS-ODN) targeting a chosen sequence of the mRNA, can arrest further translation of the gene's message and thus inhibit the particular gene product. We used the AS-ODN technology in a series of experiments in rats to demonstrate that induction of salt-dependent hypertension requires intact functional  $\alpha_{2B}$ -AR in the central SNS [54]. Rats were rendered hypertensive by subtotal nephrectomy and 1% saline as drinking water. They had then a small cannula implanted stereotaxically in the left lateral cerebral ventricle and a radiotelemetry probe for constant blood pressure and heart rate recording implanted around the aorta. Intracerebroventricular microinjection of AS-ODN targeting a chosen  $\alpha_{2B}$ -AR gene sequence in these rats produced a blood pressure lowering by  $30 \pm 5$  mmHg compared to similarly treated rats that received scrambled ODN injection. This effect on blood pressure was accompanied by a lowering of heart rate by  $82 \pm 15$  bpm and behavioral changes (drowsiness, delayed righting reflex, loss of balance), peaking at 3–6 h after injection and wearing off gradually thereafter. By 24 h these parameters had returned to baseline, at which point a second injection of the same AS-ODN produced identical results, whereas repeat injection of scrambled ODN produced no changes. In fact, in a more recent set of experiments we found that if salt-loading lasts for several days until BP becomes stabilized at a higher level, a single AS-ODN microinjection targeting the  $\alpha_{2B}$ -AR gene produces a BP lowering lasting for over 48 h. Figure 1 shows one such experiment. Injection of fluorescein-labeled AS-ODN revealed selectively increased uptake of fluorescence by several paraventricular and brainstem structures, including the optic nerve, the NTS, the locus coeruleus, as well as the cerebellum [54], all areas with a high density of  $\alpha_{2B}$ -AR. These observations corroborate the notion that induction of hypertension by salt loading is mediated by  $\alpha_{2B}$ -AR located in central SNS neurons known to be involved in blood pressure regulation and baroreflex control. Although the duration of the effect of this gene treatment with naked AS-ODN delivered directly into the CNS was very short, the use of AS-ODN carriers, such as adeno-associated viruses, can prolong the effect for up to several months, as was shown with translational inhibition of other receptors, such as the  $AT_1$  receptor of angiotensin II and the  $\beta_1$ -adrenergic receptor [53].

In another study, we sought to demonstrate the effect of translational inhibition of the  $\alpha_{2A}$ -AR gene. Since

this  $\alpha_2$ -AR subtype has a sympathoinhibitory (hypotensive) action, blocking the generation of the receptor should increase the BP. Indeed, when AS-ODN targeting a chosen sequence of the  $\alpha_{2A}$ -AR gene were microinjected in the lateral cerebral ventricle of rats, they produced an increase in BP lasting for several days. Figure 2 shows a representative individual BP recording of an AS-ODN-injected rat.

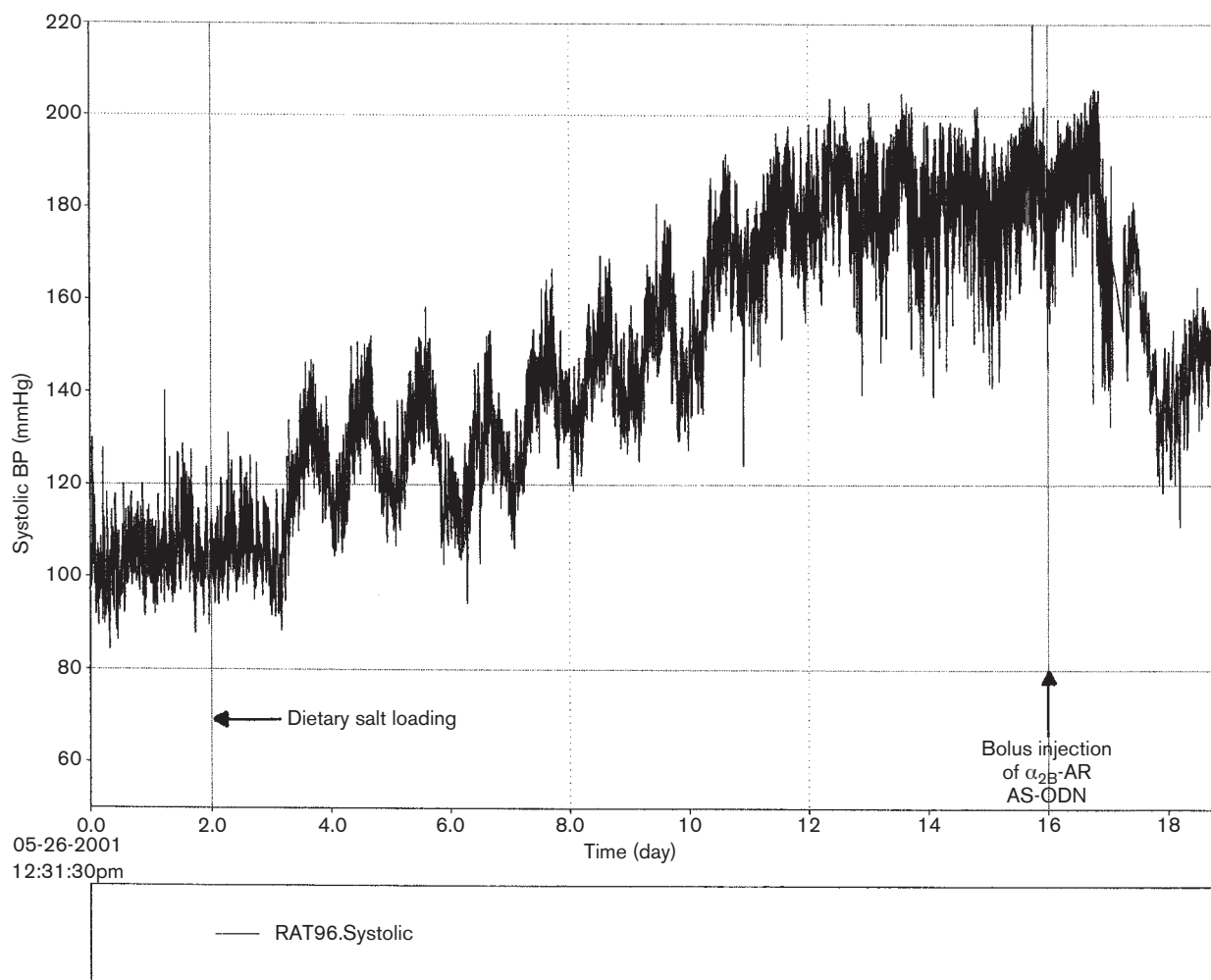
These rat experiments further reinforce the conclusions drawn from the studies with genetically engineered mice, i.e. that the central presynaptic  $\alpha_{2B}$ -AR is responsible for the hypertensive, hyperadrenergic response to salt loading, whereas the  $\alpha_{2A}$ -AR presynaptically (in the central SNS) has a sympathoinhibitory function; the postsynaptic  $\alpha_{2A}$ -AR located on the vascular wall appears to have a vasoconstrictive action.

### Clinical applications of $\alpha_2$ -AR pharmacology

One of the earliest approaches to antihypertensive therapy, introduced 35 years ago, was sympathetic suppression with clonidine, which was also shown to improve vagal tone [55]. As a highly lipophilic  $\alpha_2$ -AR agonist, clonidine acts on central sympathetic neurons, accentuating their sympathoinhibitory function, thus leading to a decrease in norepinephrine release and sympathetic nerve activity and to an overall reduction of sympathetic tone. A common characteristic of clonidine and other newly developed  $\alpha_2$ -AR agonists, such as moxonidine, rilmenidine, etc., is that all contain an imidazole ring and bind also to imidazoline and  $H_2$ -type histamine receptors with different degrees of affinity. Clonidine remains the prototype of this class of agents and its pharmacologic effects – namely, dose-dependent sympathetic suppression and decrease of circulating levels of norepinephrine – can be fully explained by its non-selective  $\alpha_2$ -AR agonistic activity on central SNS neurons, where the sympathoinhibitory  $\alpha_{2A}$ -AR is the predominant  $\alpha_2$ -AR subtype.

The antihypertensive usefulness of clonidine is well established and need not be elaborated here. Suffice it to say that it is of particular value in cases where blood pressure elevation is attributable to sudden sympathetic discharge; these include cases of extremely labile hypertension, 'neurogenic' hypertension resulting from damage of baroreflex centers of the brainstem due to hypoxia, cerebral infarcts or other lesions, hypertensive crises resulting from acute drug withdrawal in drug addicts, perioperative or intraoperative sympathetic excitation due to anxiety, hypoxia, intubation for mechanical ventilation, etc. Indeed, these conditions can mimic a pheochromocytoma crisis and the 'clonidine suppression test' can quickly differentiate excessive sympathetic discharge of CNS origin from autonomous oversecretion of catecholamines by a tumor. Clonidine, with its central vasodepressor and peripheral mild

Fig. 1



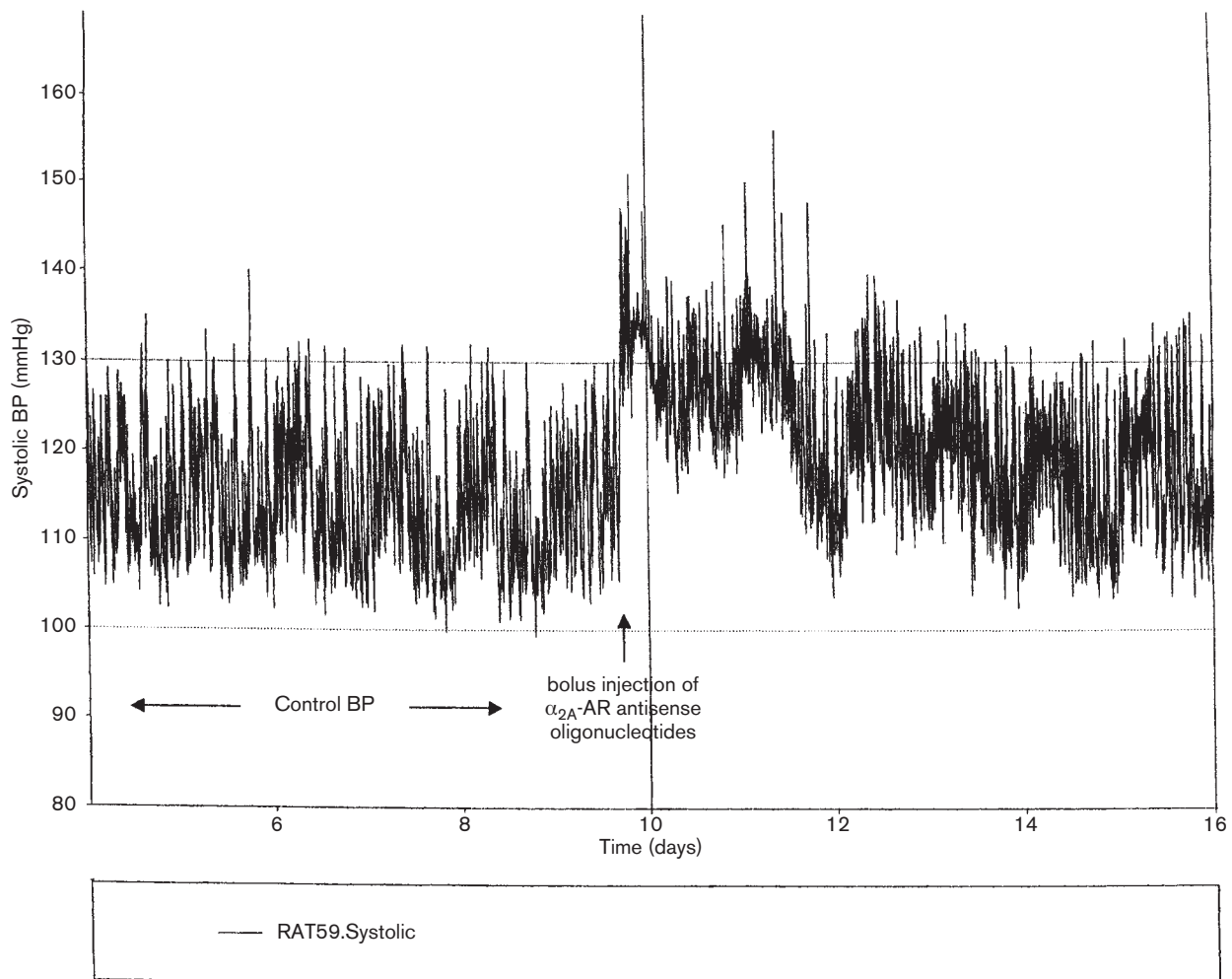
Representative individual blood pressure (BP) recording of a rat submitted to subtotal nephrectomy followed by dietary salt loading. When BP had stabilized at a hypertensive level, a single intracerebroventricular microinjection of antisense oligodeoxynucleotides (AS-ODN) targeting the  $\alpha_{2B}$ -AR gene produced a hypotensive effect lasting for over 48 h.

vasopressor effect, is particularly useful in the treatment of orthostatic hypotension with recumbent hypertension, a vexing clinical dilemma. Notably, clonidine is the only antihypertensive agent suitable for chronic transdermal administration and therefore useful when gastrointestinal absorption is unreliable, e.g. in diabetic autonomic neuropathy with gastroparesis. Interestingly, in experimental animal studies, it was found that clonidine could prevent the sympathetically mediated adverse effects of overfeeding, i.e. hypertension, tachycardia and accentuated insulin resistance, even though it did not prevent the development of obesity [56].

Another use of clonidine that we have been proposing in recent years is for the treatment of chronic or congestive heart failure (CHF) and/or ischemic heart disease, with or without hypertension. Neurohormonal activation with elevation of circulating renin, catecho-

lamines and vasopressin is characteristic of decompensated CHF [57] and is both the result of decompensation and the cause of further structural and functional deterioration. The place of inhibition of the renin-angiotensin system in the treatment of CHF has now been universally recognized; inhibition of various components of the SNS has been attempted by various means, but the results have not been uniformly beneficial. The earliest approach was  $\alpha_1$ -AR blockade, which produced a transient functional improvement followed by reversal attributed to tachyphylaxis [58]. The long-term benefits of  $\beta$ -AR blockade are now well established, provided their doses are slowly titrated and patients are carefully chosen, in order to exclude those unable to tolerate the substantial negative inotropic effect of these drugs [59], as well as the non-cardiac side-effects (e.g. bronchoconstriction).

Fig. 2



Representative individual blood pressure (BP) recording of an intact normotensive rat. After several days of recording normal BPs, a single intracerebroventricular microinjection of antisense oligodeoxynucleotides targeting the  $\alpha_{2A}$ -AR gene produced a hypertensive effect lasting over 48 h.

Clonidine had been used tentatively to treat myocardial ischemia and CHF in the past, with variable results [60,61]. It was later dismissed on the mistaken perception that it exerted a potentially harmful negative inotropic action [60], at a time when positive inotropy was believed to be necessary for treatment of CHF. Advances in knowledge of the pathophysiology of CHF in recent years have led to better understanding of the mechanisms of cardioprotection and the reasons why myocardial stimulants, despite immediate symptomatic improvement, do not benefit CHF in the long run; indeed, withdrawal of the positive inotropic and chronotropic influence of angiotensin [62], along with inhibition of its other adverse effects on cardiac tissues, is the only treatment proven incontrovertibly so far to reduce morbidity and mortality in these patients. The same appears to be true for inhibition of the effects of

norepinephrine by  $\beta$ -AR blockade in CHF patients who have no contraindication to  $\beta$ -blockers [63].

According to this line of reasoning, we hypothesized that central sympathetic suppression by clonidine should offer similar benefits deriving from the withdrawal of a positive inotropic/chronotropic/trophic stimulus. We undertook a series of small clinical trials, including acute studies conducted during diagnostic cardiac catheterization and chronic studies with repeat exercise tolerance tests and Holter monitoring over a period of several months. In acute studies of CHF lasting for up to 1 week, we found that addition of clonidine to standard therapy is very well tolerated, even by non-hypertensive patients. The profound suppression of norepinephrine (to about one-third of baseline levels) produces a small decrease in systemic BP,

but large decreases in mean right atrial pressure, pulmonary wedge pressure and pulmonary artery pressure; a pronounced increase in stroke volume is accompanied by decreased heart rate, resulting in no significant increase in cardiac output, but diminished myocardial energy demand. Not surprisingly, patients with evidence of more advanced cardiac decompensation, as indicated by baseline norepinephrine levels  $> 400$  pg/ml and poorer baseline hemodynamic parameters, were found to derive the greatest benefits from this treatment [64]. We followed a small group of such patients on this treatment for almost 2 years and evaluated them at 6 weeks and 6 months by echocardiography, ambulatory ECG (Holter) monitoring and exercise tolerance test. The suppression of norepinephrine remained constant and well tolerated; it was associated with a significant decrease in left ventricular dilation, with an average 7% increase in ejection fraction (from 32 to 39%) and increase in the duration of exercise tolerance by 88%, as well as improved parameters of heart rate variability accompanied by diminished numbers of isolated premature contractions, couplets and episodes of non-sustained ventricular tachycardia [65]. Thus, there were benefits in terms of both immediate symptomatic relief, amelioration in hemodynamic parameters, electrophysiologic stability and hormonal profile, as well as long-term improvement of functional capacity and propensity to complex arrhythmias. Moreover, the hemodynamic benefits of sympathetic suppression and angiotensin-converting enzyme inhibition seem to be additive, as shown by a trial with combination of clonidine and captopril [66], since the former improves mostly preload parameters, whereas the latter affects mostly the afterload.

In another placebo-controlled study, we added clonidine to standard therapy (including thrombolysis) in patients with acute anterior myocardial infarct (MI) and followed them for 1 week. The stress of acute MI is also characterized by a pronounced neuroendocrine activation that further contributes to clinical deterioration [67]. Compared to the placebo group, the clonidine group had a more profound and rapid suppression of neurohormones after MI, a better ejection fraction at 1 week and significantly better parameters of heart rate variability accompanied by fewer episodes of arrhythmias [68]. Notably, three patients died within the first 48 h, all from the control group and all having exhibited a progressive increase in norepinephrine, plasma renin activity and vasopressin levels after the acute MI.

Chronic hypertensive/ischemic heart disease has less dramatic manifestations and may continue for many years without clinically overt symptoms and signs of left ventricular dysfunction. However, subtle signs of diastolic dysfunction due to left ventricular wall stiffness are detectable by assessment of the pressure–

volume relation. In 10 hypertensive and 10 matched normotensive patients undergoing diagnostic cardiac catheterization, we conducted a number of hemodynamic measurements and calculated a stiffness constant and a time constant of relaxation. All 10 hypertensives were classified as having diastolic dysfunction according to these criteria and in all of them a single oral dose of 0.125 mg clonidine brought these parameters to near-normal levels [69]. Table 1 summarizes the hemodynamic and humoral responses to acute and chronic treatment with clonidine in patients with heart failure and/or ischemic heart disease. It should be noted that these patients were either normotensives or hypertensives already receiving conventional antihypertensive therapy.

Of course, evidence from long-term randomized multicenter outcome trials would be necessary to establish the validity of chronic sympathetic suppression with clonidine as a treatment capable of diminishing morbidity and mortality from ischemic heart disease and/or heart failure; unfortunately, such evidence is not yet available and may not be forthcoming for reasons unrelated to the therapeutic value of this inexpensive drug that is long known and readily available [70]. However, its safety has prompted several other investigators to test it and confirm its cardioprotective potential under various conditions, including CHF, myocardial ischemia, arrhythmogenic influences and perioperative stress [71–74]. In a recent report, clonidine was administered transdermally via a weekly patch to normotensive patients with CHF. The reduction in central sympathetic outflow, ascertained by the decrease in plasma catecholamines as well as by micro-neurography, did not interfere with normal baroreflexes and caused only minimal, insignificant changes in blood pressure and heart rate [75]. From these data we conclude that central sympathetic suppression with  $\alpha_2$ -AR agonists, such as clonidine, appears to be a valuable therapeutic tool in such conditions. In fact, suppression of the central SNS should be a preferable alternative for long-term treatment of these conditions in patients unable to tolerate  $\beta$ -adrenergic blockade due to cardiac or non-cardiac (dysmetabolic syndrome, bronchoconstriction) reasons.

### Future directions

Notwithstanding its benefits, clonidine is associated with adverse effects on psychosocial functions (such as drowsiness, diminished mental alertness and impotence), which may be acceptable in patients who are disabled from symptoms of CHF, but which limit its tolerability by relatively healthy patients with an active professional and social life. One attempt to separate the  $\alpha_2$ -AR-mediated suppression of catecholamines from these adverse effects was via development of refined ‘second-generation’ imidazole compounds (moxonidine,

**Table 1 Average early and late hemodynamic, functional and hormonal changes from baseline in response to clonidine in patients with heart failure and/or ischemic heart disease**

	24 h	7 days	6 weeks	6 months
MAP (mmHg)	-8	-4	-4	-5
RAP (mmHg)	-2.5	0		
PCWP (mmHg)	-5	-3		
HR (bpm)	-11	-9	-7	-7
CO (l/min)	+0.04	+0.07		
SVI (ml/m <sup>2</sup> )	+5	+4		
SVR (dynes s/cm <sup>5</sup> )	-123	-80		
EF (%)			+3	+7
LVSD (mm)			-1	-4
LVDD (mm)			-2	-4
SDNN (ms)			+19	+18
LV stiffness constant (/ml)	-0.0098*			
E/A ratio	+0.30*			
ETT duration (s)			+116 (+47%)	+213 (+88%)
PRA (ng/ml per h)	-0.42	-0.62	-2.9	-0.5
AVP (pg/ml)	+0.21	-0.17	+0.30	+0.90
NEPI (pg/ml)	-120	-190	-130	-150
EPI (pg/ml)	-10	-20	-28	-20

Changes within 1 h after clonidine during diagnostic cardiac catheterization. MAP, mean arterial pressure; RAP, right atrial pressure; PCWP, pulmonary capillary wedge pressure; HR, heart rate; CO, cardiac output; SVI, stroke volume index; SVR, systemic vascular resistance; EF, ejection fraction; LVSD, left ventricular diameter in systole; LVDD, left ventricular diameter in diastole; SDNN, standard deviation of normal R-R intervals (an index of heart rate variability which, if below 50 ms, indicates vagal suppression with increased propensity to ventricular arrhythmias). LV stiffness constant = left ventricular stiffness constant, calculated from the intraventricular pressures in systole and diastole, as well as the time constant for relaxation. E/A ratio (where E = peak early and A = peak late diastolic velocities) is normally > 1; a ratio of < 1 indicates diastolic dysfunction. ETT, exercise tolerance test; PRA, plasma renin activity; AVP, arginine-vasopressin; NEPI, norepinephrine; EPI, epinephrine.

rilmenidine, etc.) that would have a higher affinity for the imidazoline receptors than for the  $\alpha_2$ -ARs. Early trials indicated that these compounds have clinically significant antihypertensive properties [76]. However, the first clinical trial of moxonidine in CHF was unsuccessful and was abandoned prematurely.

Another approach could be the development of  $\alpha_2$ -AR subtype-specific agents, i.e. drugs that might suppress the central SNS by selectively activating the  $\alpha_{2A}$ -AR or inhibiting the  $\alpha_{2B}$ -AR. Alternatively, one can envision a future when gene treatment to selectively enhance or block expression of one  $\alpha_2$ -AR subtype gene may produce long-lasting therapeutic results without concurrent side-effects from interference with the functions of other subtypes.

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