Imidazoline receptors in the heart: a novel target and a novel mechanism of action that involves atrial natriuretic peptides

S. Mukaddam-Daher and J. Gutkowska
Laboratory of Cardiovascular Biochemistry, Centre Hospitalier de L’Université de Montréal Research Center, Campus Hotel-Dieu, and Department of Medicine, University of Montreal, Montreal, Quebec, Canada

Abstract

Chronic stimulation of sympathetic nervous activity contributes to the development and maintenance of hypertension, leading to left ventricular hypertrophy (LVH), arrhythmias and cardiac death. Moxonidine, an imidazoline antihypertensive compound that preferentially activates imidazoline receptors in brainstem rostroventrolateral medulla, suppresses sympathetic activation and reverses LVH. We have identified imidazoline receptors in the heart atria and ventricles, and shown that atrial I1-receptors are up-regulated in spontaneously hypertensive rats (SHR), and ventricular I1-receptors are up-regulated in hamster and human heart failure. Furthermore, cardiac I1-receptor binding decreased after chronic in vivo exposure to moxonidine. These studies implied that cardiac I1-receptors are involved in cardiovascular regulation. The presence of I1-receptors in the heart, the primary site of production of natriuretic peptides, atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP), cardiac hormones implicated in blood pressure control and cardioprotection, led us to propose that ANP may be involved in the actions of moxonidine. In fact, acute iv administration of moxonidine (50 to 150 µg/rat) dose-dependently decreased blood pressure, stimulated diuresis and natriuresis and increased plasma ANP and its second messenger, cGMP. Chronic SHR treatment with moxonidine (0, 60 and 120 µg kg⁻¹ h⁻¹, sc for 4 weeks) dose-dependently decreased blood pressure, resulted in reversal of LVH and decreased ventricular interleukin 18 concentration after 4 weeks of treatment. These effects were associated with a further increase in already elevated ANP and BNP synthesis and release (after 1 week), and normalization by 4 weeks. In conclusion, cardiac imidazoline receptors and natriuretic peptides may be involved in the acute and chronic effects of moxonidine.

Key words
- Sympathetic nerve activity
- Imidazoline receptors
- Moxonidine
- Atrial natriuretic peptide
- Brain natriuretic peptide
- Hypertension
- Heart

Correspondence
S. Mukaddam-Daher
Laboratory of Cardiovascular Biochemistry, CHUM-Research Center Campus Hotel-Dieu (6-816) 3840 St-Urbain Street Montreal, Quebec Canada, H2W 1T8 Fax: +514-412-7199 E-mail: suhayla.mukaddam-daher@umontreal.ca

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Introduction

The sympathetic nervous system (SNS) plays an important role in the regulation of blood pressure and cardiac function through the control of vasomotor tone, heart rate and the activity of the renin-angiotensin-aldosterone system. Sympathetic nervous activity is regulated in centers in the brain stem and transmitted to organs and blood vessels that are innervated by sympathetic nerve endings, modifying cardiac output, peripheral vascular resistance and renal function. Sympathetic overactivity is one of the mechanisms associated with several cardiovascular diseases, including development and maintenance of genetic hypertension. Clinical studies have shown that sympathetic innervation of cardiovascular organs is enhanced in young patients with essential hypertension, and that half of the enhanced total norepinephrine spillover - a measure of sympathetic activity, is derived from increases to the heart and kidneys (1). Hypertension-associated selective overactivity in the sympathetic nerves to the heart, kidneys and skeletal muscle vasculature, leads to development of left ventricular hypertrophy, predisposing to ventricular arrhythmias, increasing insulin resistance, and accelerating atherogenesis. Therefore, it is not surprising that most of the established cardiovascular therapy targets sympathetic nervous activity. Over the years, several types of antihypertensive drugs have been developed to inhibit SNS activity at multiple levels, including release, transmission or action. Peripheral α1-adrenergic and β-adrenergic receptor antagonists, postganglionic sympathetic neuron blockers and ganglioplegic agents, as well as central activation of α2-adrenoceptors are routine ways to reduce high blood pressure by lowering peripheral SNS activity. The important clinical benefits of angiotensin converting enzyme inhibitors and angiotensin II receptor antagonists could perhaps be explained by their blunting or inhibiting the SNS effects in patients with cardiovascular disease. In contrast, some calcium antagonists which are successfully used to treat patients with hypertension and stable angina pectoris may have unfavorable effects in patients with impaired left ventricular function, due in part to baroreceptor-mediated activation of the SNS.

Therefore, it appears imperative that selection of vasoactive drugs for the treatment of cardiovascular diseases takes into consideration their effects on the SNS, where ideally treatment should also blunt sympathetic activity, or at least avoid activating it. Centrally acting drugs, such as clonidine and α-methyldopa, reduce central sympathetic drive, and consequently maintain cardiac output, arterial tone and body fluid volume. Despite their clinical efficacy, the use of these “first generation centrally acting drugs” was often limited by their side effects, which include, dry mouth, sedation and mental depression. However, the discovery of imidazoline receptors and development of selective compounds, moxonidine and rilmenidine, which show an improved side-effect profile, revived interest in centrally acting drugs. Compared to the reference drug, clonidine, the “second generation centrally acting drugs”, moxonidine and rilmenidine, show three to ten times higher affinity for imidazoline receptors than α2-adrenoceptors (2). It may be due to this selectivity that moxonidine and rilmenidine have fewer side effects, because imidazoline receptors have been shown to mediate hypotension, while sedation was attributed to α2-adrenoceptors.

Discovery of imidazoline receptors

Bousquet and colleagues (3) identified the primary site of the hypotensive action of clonidine as the nucleus reticularis lateralis region of the rostroventrolateral medulla (RVLM), which is a major source of sympathetic vasomotor drive via the preganglionic
neurons in the spinal cord. These investigators noted that microinjections of clonidine, imidazoline and related compounds, in this area, decreased blood pressure, whereas phenylethylamine compounds did not. Also, imidazoline, but not non-imidazoline \(\alpha_2\)-adrenoceptor antagonists, prevented imidazoline-induced hypotension when administered directly into this area. Accordingly, they proposed that the hypotensive action of clonidine and related imidazoline drugs is not mediated by \(\alpha_2\)-adrenoceptors, but may involve a new binding site that recognizes the imidazoline ring structure in these compounds (4).

Pharmacologic characterization by binding assays confirmed the existence of a population of imidazoline-specific binding sites that are insensitive to catecholamines in the brainstem RVLM of animals and man (2,5,6). These studies clearly demonstrated that imidazoline binding sites are distinct from \(\alpha_2\)-adrenoceptors.

Binding studies have classified imidazoline binding sites into two main subtypes according to their affinity for clonidine (7). The imidazoline \(I_1\) sites, enriched in plasma membranes, have high affinity for \(^{3}H\)-clonidine and its analogues, whereas imidazoline \(I_2\) binding sites are sensitive to idazoxan and its analogues, but not to clonidine. Imidazoline \(I_2\) sites, which are exclusively mitochondrial, were shown to reside on monoamine oxidases (8). Further functional studies proposed the presence of non-\(I_1\)/non-\(I_2\) sites, located prejunctionally on postganglionic sympathetic nerve terminals, and \(I_3\) sites, which are implicated in insulin release from pancreatic \(\beta\) cells. The focus of our research is on the \(I_1\) subtype which has high affinity for clonidine, moxonidine and rilmenidine, and which has been implicated in cardiovascular regulation.

In a series of studies and a systematic review, Ernsberger (9) demonstrated that the imidazoline \(I_1\) binding sites fulfill the essential criteria for identification as functional receptors. The vasodepressor response to imidazoline is consistent with each of the four major criteria of specificity, function, location, and correlation of affinity with function. In addition, \(I_1\)-receptors are subject to physiologic regulation, in a manner distinct from \(\alpha_2\)-adrenoceptors and, similar to other neurotransmitter receptors, imidazoline \(I_1\) binding can be downregulated by chronic exposure to agonists (10,11). Imidazoline \(I_1\)-receptors have at least one endogenous ligand, a “clonidine displacing substance” isolated from the brain and identified by Reis and co-workers (12) as agmatine (decarboxylated arginine), previously unknown in mammals. Agmatine has been shown to be widely distributed in many tissues, including the heart.

Two mechanisms of \(I_1\)-receptor signal transduction have been identified. Ernsberger (9) demonstrated in PC12 rat pheochromocytoma cells, which lack \(\alpha_2\)-adrenoceptors, that \(I_1\) receptor signaling includes activation of phosphatidylcholine-selective phospholipase C and generation of diglycerides and the release of arachidonic acid and its metabolites. The cascade involves activation of protein kinase C and mitogen-activated protein kinase (13). Other studies have also associated imidazoline \(I_1\)-receptors to inhibition of adenylyl cyclase (14).

**Cardiac imidazoline \(I_1\)-receptors**

Imidazoline \(I_1\)-receptors have been localized in the brainstem RVLM, and in the peripheral nervous system, including adrenomedullary chromaffin cells and carotid body, as well as in kidneys. In addition, we have demonstrated the presence of imidazoline \(I_1\)-receptors in the heart. Using radiolabeled para-iodoclonidine (\(^{125}\)I-PIC, 2200 Ci/mmol; New England Nuclear, Boston, MA, USA), which binds to \(I_1\)-imidazoline and \(\alpha_2\)-adrenoceptors, both receptor types were identified in the heart of multiple species, including rat, dog, and man. After irreversible blockade of \(\alpha_2\)-adrenoceptors, \(^{125}\)I-PIC
binding to heart frozen sections and atrial and ventricular membranes was competitively inhibited by increasing concentrations (10^{-12} to 10^{-6} M) of moxonidine, but not by epinephrine, implying specific imidazoline I_1-receptor binding. Data from membrane binding and autoradiography were confirmed by immunohistochemistry and Western blotting (15).

Furthermore, we showed that heart I_1-receptors but not α2-adrenoceptors are pathophysiologically regulated. Imidazoline I_1-receptors were up-regulated in spontaneously hypertensive rat (SHR) atria. Compared to age-matched normotensive Wistar Kyoto rats, I_1-receptor Bmax was 2-fold (P < 0.01) higher in 12-week-old SHR right and left atria, but not altered in ventricles. Compared to corresponding normal controls, Bmax was 2-fold higher (P < 0.01) in ventricles of hamsters with advanced cardiomyopathy and in human ventricles with heart failure (Figure 1) (15). We also demonstrated that cardiac I_1-receptors, but not α2-adrenoceptors, are regulated by chronic in vivo exposure to the I_1-receptor selective ligand, moxonidine. SHR (12-14 weeks old) received 0, 10, 60, or 120 μg kg^{-1} h^{-1} moxonidine (a generous gift from Solvay Pharmaceuticals, Hannover, Germany), via subcutaneously implanted Alzet minipumps (2ML1 and 2ML4; Cedarlane Laboratories Ltd., Hornby, ON, Canada), for 1 and 4 weeks. This treatment resulted in a dose-dependent decrease in specific 125I-PIC binding in atria by 1 week, with no further decrease after 4 weeks. Moxonidine (120 μg kg^{-1} h^{-1}) decreased (P < 0.01) I_1-receptor Bmax in right and left atria to normal levels (Figure 2), without altering α2-adrenoceptor Bmax. These results showed for the first time that imidazoline I_1-receptors and α2-adrenoceptors are present in heart atria and ventricles, and that only imidazoline I_1-receptors are responsive to changes in the cardiovascular environment and to chronic exposure to the I_1-selective agonist, moxonidine (15,16). These results implicate heart imidazoline I_1-receptors in cardiovascular regulation.

Imidazoline and natriuretic peptides

The efficacy of antihypertensive imidazoline compounds has been well established. Acute and long-term hemodynamic studies show that moxonidine reduces arterial pressure by lowering systemic vascular resistance while sparing heart rate, cardiac output and stroke volume. The effects are selectively mediated by activation of imidazoline I_1-receptors and subsequent inhibition of sympathetic tone. Inhibition of renal sympathetetic activity and/or direct activation of kidney imidazoline I_1-receptors induce diuresis and natriuresis (17,18), thus contributing to acute and long-term blood pressure control. In addition, the benefits of imidazoline compounds go beyond blood pressure control...
through their cardioprotective effects, anti-proliferative and anti-arrhythmic, as well as their anti-inflammatory actions (19-25). Moxonidine and rilmenidine reduce left ventricular hypertrophy in patients and rats (20-22). Rilmenidine pretreatment (iv or ic) has been shown to dose-dependently prevent the occurrence of bicuculline-induced arrhythmias in anesthetized rabbits (23). Clonidine significantly decreases TNF-α production in macrophages and noradrenergic nerve terminals in in vivo lipopolysaccharide-treated mice (24). In type 2 diabetes, imidazoline compounds protect against proinflammatory interleukin 1ß-induced beta-cell apoptosis (25).

The beneficial effects of imidazoline may be centrally or peripherally mediated. However, identification of imidazoline I1-receptors and α2-adrenoceptors in the heart, mainly in cardiac atria, appeared very interesting. The atria are the primary site of atrial natriuretic peptide (ANP) production. ANP is a member of the natriuretic peptide family that also includes brain natriuretic peptide (BNP), C-type natriuretic peptide (CNP), and urodilatin, a family of peptides involved in blood pressure and volume regulation. The diuretic, natriuretic and vasodilator actions of natriuretic peptides are mediated by cell membrane natriuretic peptide receptor A (NPR-A) and NPR-B, through activation of guanylyl cyclase/cGMP, and NPR-C (clearance receptor), through inhibition of adenylyl cyclase/cAMP. The important role of ANP on chronic blood pressure regulation has been shown in a transgenic mouse model overexpressing ANP (26,27), where elevated plasma ANP was associated with significant blood pressure reduction, whereas NPR-A knockout mice are hypertensive. The knock-out studies revealed that the chronic hypotensive effect of ANP is mediated by attenuation of cardiovascular sympathetic tone (27). Independent of their role in blood pressure control, natriuretic peptides have anti-inflammatory effects, and exert direct anti-hypertrophic and anti-fibrotic actions on the heart. Mice lacking NPR-A (which preferentially binds ANP and BNP) display a marked cardiac (atrial and ventricular) hypertrophy and extensive fibrosis and chamber dilatation by 3 months of age, as well as high mortality rate due to sudden death (28,29). Mice with disrupted BNP develop multifocal fibrotic lesions in their ventricles (30). In addition, ANP has been shown to inhibit pro-inflammatory cytokine-induced nitric oxide production in human proximal tubular cells in culture (31).

Therefore, on the basis of the presence in the heart of both imidazoline receptors and natriuretic peptides, and on the functional resemblance between their actions, the following studies were performed to investigate whether activation of imidazoline I1-receptors by moxonidine may involve natriuretic peptides.

In acute studies, intravenous bolus injections of moxonidine (50 to 150 µg/rat) in conscious normotensive rats (32) and SHR (33) reduced blood pressure and dose-dependently stimulated diuresis, natriuresis, kaliuresis and increased plasma ANP, and urinary cGMP, the index of ANP activity, suggesting that ANP is involved in the acute effects of moxonidine.

In chronic in vivo studies, SHR received moxonidine at 0, 60 and 120 µg kg⁻¹ h⁻¹, over 4 weeks. Telemetric measurement of blood pressures revealed that the higher doses dose-dependently decreased systolic, diastolic and mean arterial pressures and heart rate starting on the first day, reaching a minimum by 4 days, and maintained at low levels throughout the 4-week treatment. After 4 weeks, moxonidine at 120 µg resulted in a significant reversal of left ventricular hypertrophy measured as the ratio of left ventricular weight to body weight (Figure 3). Also, in these hearts, moxonidine decreased elevated interleukin 1ß levels (ELISA Kit, Biosource International Inc., Camarillo, CA, USA) in left ventricles from 166 ± 4 to 119 ± 11 pg/mg protein (P < 0.01) (Figure 4). Most im-
portantly, the effects were associated with increased atrial synthesis and release of natriuretic peptides, ANP and BNP (Figure 5), after 1 week, and normalization by 4 weeks of treatment (34,35). Since natriuretic peptides and their receptors are present in the heart, we propose that moxonidine-stimulated ANP may act in a paracrine/autocrine manner to oppose the inflammatory and hypertrophic effect of overactive SNS and hypertension-associated cardiovascular environment.

We have not investigated the mechanism(s) of ANP production by moxonidine. The increase in ANP may be explained in part by moxonidine inhibition of sympathetic drive to the heart. Alternatively, imidazoline receptors and/or α2-adrenoceptors present in the heart may directly or indirectly affect ANP release from cardiac myocytes and/or ANP granules identified in the conduction system. Further studies are required to investigate the mechanism(s) of ANP release by moxonidine.

Physiological significance

It appears that the overall actions of the natriuretic peptide system are similar to, and most likely mediate, the effects of imidazoline receptor agonists. Increased natriuretic peptides may contribute to the therapeutic mechanism of imidazoline receptor agonists through their sympatholytic, anti-inflammatory, anti-fibrotic, and anti-proliferative effects. Accordingly, the use of drugs that potentiate endogenous natriuretic peptides, such as imidazoline receptor activators, should be favored. In addition, heart I1-receptors should be considered a target for antihypertensive treatment.

References


