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The I₁-imidazoline receptor: from binding site to therapeutic target in cardiovascular disease

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Abstract

Objective To review previous work and present additional evidence characterizing the I₁-imidazoline receptor and its role in cellular signaling, central cardiovascular control, and the treatment of metabolic syndromes. Second-generation centrally-acting antihypertensives inhibit sympathetic activity mainly via imidazoline receptors, whereas first-generation agents act via α_2 -adrenergic receptors. The I₁ subtype of imidazoline receptor resides in the plasma membrane and binds central antihypertensives with high affinity.

Methods and results Radioligand binding assays have characterized I₁-imidazoline sites in the brainstem site of action for these agents in the rostral ventrolateral medulla. Binding affinity at I₁-imidazoline sites, but not at other classes of imidazoline binding sites, correlates closely with the potency of central antihypertensive agents in animals and in human clinical trials. The antihypertensive action of systemic moxonidine is eliminated by the I₁/ α_2 -antagonist efaroxan, but not by selective blockade of α_2 -adrenergic receptors. Until now, the cell signaling pathway coupled to I₁-imidazoline receptors was unknown. Using a model system lacking α_2 -adrenergic receptors (PC12 pheochromocytoma cells) we have found that moxonidine acts as an agonist at the cell level and I₁-imidazoline receptor activation leads to the production of the second messenger diacylglycerol, most likely through direct activation of phosphatidylcholine-selective phospholipase C. The obese spontaneously hypertensive rat (SHR; SHROB strain) shows many of the abnormalities that cluster in human syndrome X, including elevations in blood pressure, serum lipids and insulin. SHROB and their lean SHR littermates were treated with moxonidine at 8 mg/kg per day. SHROB and SHR treated with moxonidine showed not only lowered blood pressure but also improved glucose tolerance and facilitated insulin secretion in response to a glucose load. Because α_2 -adrenergic agonists impair glucose tolerance, I₁-imidazoline receptors may contribute to the multiple beneficial effects of moxonidine treatment.

Conclusion The I₁-imidazoline receptor is a specific high-affinity binding site corresponding to a functional cell-surface receptor mediating the antihypertensive actions of moxonidine and other second-generation centrally-acting agents, and may play a role in countering insulin resistance in an animal model of metabolic syndrome X.

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Keywords

central cardiovascular control; imidazoline receptors; insulin resistance; moxonidine; radioligand binding; rostral ventrolateral medulla; obese spontaneously hypertensive rats

Introduction

Selective inhibition of the sympathetic nervous system (SNS) is an effective means of lowering blood pressure, treating congestive heart failure, and preventing recurrence of myocardial infarction. Beginning with the introduction of reserpine in the 1950s, selective agents have been developed to act within the central nervous system, in autonomic ganglia, in peripheral nerves and at catecholamine receptors throughout the body [1]. Currently, the most popular means of attenuating SNS activity is by blockade of systemic β - and α_1 -adrenergic receptors. The primary advantage of catecholamine receptor blockade is the reduced incidence of side-effects relative to the first generation of SNS inhibitors which included guanethidine, α -methyldopa, and clonidine. However, attenuation of SNS activity through the CNS has advantages over systemic catecholamine receptor blockade. Stimulation of α - and β -adrenergic responses are reduced in parallel by reduction of catecholamine supply, whereas β -blockade leads to unopposed α -adrenergic stimulation. Central sympatholytic agents have the advantage of preserving normal physiological activation of SNS activity during postural adjustments, exercise and hypercapnia [2]. Importantly, activation of SNS activity during psychological stress is attenuated after treatment with imidazoline sympatholytic agents in humans [3] and in animals [4–6]. Excess catecholamines have direct deleterious effects on cardiac hypertrophy, arrhythmias, and vascular disease [7]. Furthermore, SNS overactivity decreases glucose tolerance and may exacerbate non-insulin-dependent diabetes [4,8]. Therefore, inhibition of SNS activity may be effective for any of the indications for which β -blockers or α_1 -adrenergic antagonists are used. Moreover, selective central blockade of SNS activity may be effective against other cardiovascular and metabolic conditions that commonly accompany hypertension.

In the recent past, centrally acting SNS inhibitors were seldom used because of unpleasant side-effects such as sedation and dry mouth [1], yet the predominant means of attenuating the actions of the SNS was to give systemic β -adrenergic blockers. Today there is a resurgence of interest in centrally acting agents because a second generation of central sympatholytics has appeared, represented by rilmenidine [9,10] and moxonidine [11]. These second-generation agents lack the side-effects produced by the first generation of SNS inhibitors, such as α -methyldopa, guanabenz and clonidine, which resulted from the activation of brain α_2 -adrenergic receptors. Although retaining some activity at α_2 -adrenergic receptors, and thus some potential for α_2 -mediated side-effects, rilmenidine and moxonidine inhibit SNS activity through a new mechanism mediated by a novel receptor type specific for imidazolines. Clonidine also binds to and activates these I_1 -imidazoline receptors with high affinity and potency. However, clonidine has much higher affinity for α_2 -adrenergic receptors than rilmenidine or moxonidine, which correlates with its adverse side-effect profile.

In this article, we will survey recent findings on I_1 -imidazoline sites and their functional role at the cellular and organismic levels. Beginning from initial observations regarding a binding site, we outline our present understanding of the I_1 -imidazoline receptor as a cell-surface receptor mediating cellular responses, participating in cardiovascular control by the CNS, and providing a potential therapeutic target for multiple cardiovascular and metabolic disorders.

Radioligand binding studies of I₁-imidazoline sites

The first experiments demonstrating imidazoline-specific binding sites in the brainstem are depicted in Fig. 1, replotted and reanalyzed from the original 1987 report [12]. These studies characterized the binding of [³H]-p-NH₂-clonidine to cell membranes obtained from the rostral ventrolateral medulla (RVLM), which has been identified as the site of action for imidazoline antihypertensive agents such as clonidine [13], rilmenidine [9,10] and moxonidine [14,15]. Norepinephrine could inhibit only 64% of total specific binding sites for [³H]-p-NH₂-clonidine in the RVLM, whereas unlabeled clonidine inhibited the full complement of sites. These data show that 36% of specific binding sites in the RVLM are non-adrenergic, yet retain high affinity for imidazolines such as clonidine.

Also displayed in Fig. 1 are replotted and reanalyzed data from a subsequent report from the Strasbourg group [16] using bovine RVLM membranes but with [³H]-clonidine as the radioligand. Despite methodological differences between the two reports [12,16], the data are nearly superimposable. Norepinephrine inhibited only 64 and 69% of the total specific binding of [³H]-p-NH₂-clonidine and [³H]-clonidine, respectively. Unlabeled clonidine bound to all of the sites with high affinity, including the non-adrenergic subpopulation, with almost perfectly identical results [12,16]. Furthermore, the two studies found nearly identical K_D values (6 versus 3 nM). Two additional groups have repeated the experiment shown in Fig. 1 and have also reported nearly superimposable results [17,18]. Moreover, several other groups have reported high-affinity imidazoline sites with properties similar to I₁-imidazoline receptors [19–21].

We have used the method of non-linear curve-fitting (Figs 1 and 2), to test a series of compounds for their relative affinities at I₁-imidazoline and α₂-adrenergic receptors in bovine RVLM membranes. The antihypertensive agents with an imidazoline or related structure all show high affinity for I₁-imidazoline sites (affinity constant K_i <10 nM; Table 1). The second-generation agents with reduced sedative side-effects, moxonidine and rilmenidine, show lower affinity (higher K_i) at α₂-adrenergic receptors relative to first-generation agents with pronounced sedative side-effects, including clonidine, guanabenz, guanfacine, and α-methylnorepinephrine (the active metabolite of α-methyldopa). The ratio of affinity constants for I₁-imidazoline receptors to those for α₂-adrenergic receptors provides an index of selectivity for each compound which clearly distinguishes first- and second-generation agents. Paralleling the development of rilmenidine and moxonidine as selective centrally-acting antihypertensive agents with reduced sedative and CNS depressant activity, a new class of selective α₂-adrenergic agonists has been developed as sedatives and anesthetic/analgesic agents which lack major cardio-vascular actions. Thus, D-medetomidine has been introduced as a veterinary anesthetic agent which allows rapid recovery following the administration of a selective α₂-adrenergic antagonist [22]. Because D-medetomidine is an imidazole, some investigators have speculated that this compound may interact with I₁-imidazoline receptors [23,24]. Medetomidine (the racemate of D-medetomidine) and its analog detomidine bind with high affinity to about one-half of the specific sites labeled by [³H]-p-NH₂-clonidine in the RVLM (Fig. 2), whereas very high concentrations of these agents are required to inhibit the remaining half. Non-linear curve-fitting analysis (Table 1) confirms the very high affinity of detomidine and medetomidine for α₂-adrenergic receptors in the brainstem and shows the low affinity of these anesthetic/analgesic agents for I₁-imidazoline receptors. This result confirms similar findings in human platelets [25]. Therefore, medetomidine is a highly selective α₂-adrenergic agonist, which probably accounts for the ability of this agent to induce profound sedation and anesthesia with only modest declines in blood pressure.

Recent reports from the Strasbourg group have described imidazoline-specific binding sites labeled with [³H]-clonidine that differ from I₁-imidazoline receptors in several respects. Most

strikingly, these imidazoline sites have lower affinity for clonidine, rilmenidine and moxonidine. These low-affinity sites are only occupied when micromolar concentrations of these drugs are present. Given that the peak plasma concentrations of the imidazoline antihypertensive drugs are below 10 nM, it is unlikely that these low-affinity imidazoline sites are active under therapeutic conditions. The low-affinity imidazoline sites are insensitive to guanine nucleotides, raising the possibility that they could be an uncoupled or latent form of the I₁-imidazoline receptor. Another possibility is that low-affinity sites may represent a truncated or partially proteolyzed form of the I₁-imidazoline receptor. In support of this speculation, the ratio of high-affinity to low-affinity imidazoline sites is increased with the inclusion of multiple protease inhibitors [26]. Other investigators have reported imidazoline binding sites with affinities in the micromolar range in brain and kidney [27,28].

Binding sites with the characteristics of high-affinity I₁-imidazoline sites have been described in the medulla oblongata of the cow [12,29], and the rat [17,30]. These sites have also been characterized in the peripheral nervous system, including chromaffin cells from the adrenal medulla [19,31,32] and in tumor cells derived from this organ (PC12 pheochromocytoma cells) [33,34]. Glial cells in the brain lack imidazoline sites and express only α_2 -adrenergic receptors [35], implying that within the brain I₁-imidazoline sites are localized to neurons.

Cellular signaling pathways coupled to I₁-imidazoline receptors

The I₁-imidazoline site is selectively localized to cell fractions enriched in plasma membranes from human platelets [25], the RVLM [36], and PC12 pheochromocytoma cells [34]. Therefore, I₁-imidazoline binding sites are likely to be cell-surface proteins, in contrast to the mitochondrial location of I₂-imidazoline sites [37,38].

At receptors coupled to guanine nucleotide binding regulator proteins, or G proteins, the addition of guanine nucleotides attenuates the binding of agonists but not antagonists. Previous reports have shown that guanine nucleotides, such as GTP and its analogs, inhibit the binding of I₁-imidazoline agonist radioligands in human platelets and bovine adrenomedullary chromaffin cells [25,33,34]. In bovine RVLM and in canine prostate, guanine nucleotides inhibit [³H]-clonidine binding even after α_2 -adrenergic receptors have been irreversibly inactivated [36,39]. Considered together, these data imply that I₁-imidazoline sites may be coupled to a G protein.

The plasma membrane localization and guanine nucleotide sensitivity of I₁-imidazoline binding sites imply that they may be functional receptors coupled to cellular responses. In order to determine signaling pathways coupled to I₁-imidazoline receptors, PC12 pheochromocytoma cells have been identified as a useful model. This tumor cell line expresses I₁-imidazoline receptors, but lacks α_2 -adrenergic receptors as shown by the absence of α_2 -binding sites [34,40] and the failure to detect mRNA for this receptor [41]. Therefore, unlike the cardiovascular functional tests described below, responses to I₁-imidazoline agonists can be tested directly without prior blockade of α_2 -adrenergic receptors. Several cellular response to the stimulation of I₁-imidazoline receptors have been described in PC12 cells, including the release of prostaglandin E₂ [34] and arachidonic acid [42]. Stimulation of I₁-imidazoline receptors increases the formation of diacylglyceride from phospholipids and elevates the total cellular mass of this second messenger [40]. We have recently shown [40] that diacylglyceride production in PC12 cells elicited by moxonidine was competitively inhibited by efaroxan, a putative I₁-imidazoline antagonist [15,43]. The accumulation of diacylglyceride was not accompanied by activation of phospholipase D [40]. Previous studies have also ruled out activation of the phosphatidylinositol-selective phospholipase C signaling system by I₁-imidazoline receptors [44–46]. By exclusion, these findings implicated phosphatidylcholine

selective-phospholipase C (PC-PLC) in the accumulation of diacylglyceride in response to activation of I₁-imidazoline receptors.

Our most recent cell signaling studies provide further evidence that I₁-imidazoline receptors are coupled to PC-PLC in PC12 cells *in vitro* and further that I₁-imidazoline receptors in the RVLM *in vivo* act to lower blood pressure through activation of PC-PLC [47]. In PC12 cells, moxonidine increased the generation of two reaction products induced by PC-PLC: diacylglyceride and phosphocholine. The appearance of both products was prevented by treatment with D609, a specific inhibitor of PC-PLC [48,49]. In SHR, we observed an abolition of the vasodepressor response to intravenous moxonidine (40 µg/kg) by microinjection of D609 in the RVLM, implying that an intact PC-PLC pathway in the RVLM is required for the antihypertensive action of moxonidine [47]. These data also provide further support for the localization of the vasodepressor response to systemic moxonidine within the RVLM [15].

A hypothetical model for the signaling pathway activated by I₁-imidazoline receptors in PC12 cells and in the RVLM is depicted in Fig. 3. Binding of the agonist moxonidine to the I₁-imidazoline receptor leads to activation of PC-PLC, possibly through coupling to an unidentified G protein, as suggested for other receptor systems coupled to PC-PLC. The plasma membrane enzyme PC-PLC, in turn, uses phosphatidylcholine as a substrate and generates diacylglyceride and phosphocholine. Besides the accumulation of the lipid second messenger diacylglyceride, we have also shown that I₁-receptor stimulation in PC12 cells elicits the release of prostaglandins [34] and their precursor, free arachidonic acid [42]. One pathway that may link the accumulation of diacylglyceride to an increase in arachidonic acid release is diacylglyceride lipase. Also indicated in Fig. 3 are the inhibitors D609, blocking PC-PLC, and efaroxan and BDF-6143, competitive antagonists acting at the I₁-imidazoline receptor *in vitro* [40] and *in vivo* [15,43]. Even though the physiological role of agonist-stimulated PC-PLC is not completely understood [50], our findings suggest that PC-PLC may participate in the regulation of blood pressure by the RVLM.

Role of I₁-imidazoline receptors in vasodepressor responses to moxonidine and other imidazolines

The primary criterion for the identification of a binding site as a functional receptor is a one-to-one correspondence of binding affinities to relative potencies in eliciting functional drug responses [51]. This criterion has been met by I₁-imidazoline receptors for the following functional responses: (1) the vasodepressor response to direct microinjection into the RVLM of anesthetized Sprague–Dawley rats [29,52], (2) the vasodepressor response to intracisternal injections in conscious SHR [53], (3) antihypertensive activity in human clinical trials [54], and (4) the induction in adrenal chromaffin cells of messenger RNA encoding the synthetic enzyme for epinephrine (phenylethanolamine N-methyltransferase) [55]. An example is shown in Fig. 4a. The relative binding affinities of seven different imidazolines and related compounds for I₁-imidazoline receptors in the bovine RVLM significantly correlated with their ability to lower blood pressure when microinjected into the RVLM of Sprague-Dawley rats. This correlation supports the hypothesis that I₁-imidazoline binding sites represent functional receptors. The relationship between I₁-imidazoline binding affinity and vasodepressor activity is independent of α₂-adrenergic receptors, because α₂-adrenergic affinity was unrelated to vasodepressor action ($r = -0.16$ [52]). Furthermore, cimetidine, a histamine H₂ antagonist and I₁-imidazoline agonist [34], lowered blood pressure to an extent consistent with its I₁-affinity (Fig. 4a) despite a lack of affinity for α₂-adrenergic receptors.

In order to test the hypothesis that the low-affinity imidazoline sites described by the Strasbourg group and others are also functional receptors, we performed a correlational analysis of reported binding affinities [16,56] with vasodepressor activity (Fig. 4b). There was no significant

relationship between these two variables ($r = -0.18$). Several agents that effectively lowered blood pressure, including p-NH₂-clonidine, moxonidine and cimetidine, showed extremely low affinity for these sites. Furthermore, whereas binding affinity at I₁-imidazoline receptors correlates strongly with clinical doses required for antihypertensive efficacy in human hypertension ($r = 0.996$, $n = 4$) [54], there is no relationship between antihypertensive efficacy and binding activity at low-affinity imidazoline sites ($r = 0.258$, $n = 3$). Thus, clonidine and rilmenidine have similar affinities [56], but the former agent is more efficacious by a factor of 7. Similarly, moxonidine is completely inactive at low-affinity sites, yet moxonidine shows an efficacy comparable to clonidine. The conclusion must therefore be made that low-affinity imidazoline sites are unlikely to participate in the central regulation of blood pressure, because binding affinity is unrelated to antihypertensive efficacy, and because the concentrations of drugs required for occupancy of these sites greatly exceed therapeutic levels. The involvement of imidazoline receptors in antihypertensive actions therefore appears to be confined to the high-affinity form of the I₁-subtype.

The demonstration of a direct linear relationship between I₁-imidazoline binding affinity and vasodepressor potency supports, but does not by itself establish, the hypothesis that an I₁-imidazoline receptor contributes to vasodepressor responses to imidazolines in the RVLM. A more direct test of the I₁-imidazoline receptor hypothesis comes from studies using selective antagonists to distinguish the relative roles of I₁-imidazoline and α_2 -adrenergic receptors. At present, there are no antagonists which block I₁-imidazoline receptors without affecting α_2 -adrenergic receptors as well (Table 1). However, there are several α_2 -antagonists with almost no affinity for I₁-sites, including rauwolscine and SK&F86466. Rauwolscine, like yohimbine, is a relatively non-selective compound with equal affinity for serotonin 5-hydroxytryptamine (5HT)_{1A} and α_2 -adrenergic receptors [57–59]. For most of our studies, therefore, we have used SK&F86466, a rigid chemical analog of norepinephrine, as the most specific α_2 -antagonist available. To block I₁-imidazoline receptors, we currently use efaroan, which has moderate selectivity for I₁-imidazoline over α_2 -adrenergic receptors (Table 1). The I₁-imidazoline receptor hypothesis predicts that the I₁/ α_2 -antagonist efaroan will block the antihypertensive actions of imidazolines whereas the specific α_2 -antagonist SK&F86466 will be ineffective. In contrast, if only α_2 -adrenergic receptors were relevant in the responses to imidazolines, then efaroan and SK&F86466 should be equally effective blockers. *In vivo* studies show that efaroan but not SK&F86466 blocks the action of moxonidine in the RVLM [15].

Several recent studies have reported contrary or mixed results and have argued against the I₁-imidazoline receptor hypothesis. The primary argument in favor of the hypothesis that clonidine and other imidazoline centrally-acting antihypertensives reduce blood pressure through an activation of α_2 -adrenergic receptors is that the hypotensive effect of clonidine can be blocked by α_2 -adrenergic antagonists. However, there are several weaknesses in these experiments that have become apparent as more knowledge has accumulated. First, many of the antagonists have significant affinity for I₁-imidazoline sites. This is obviously true for the imidazoline antagonists such as idazoxan, methoxyidazoxan (Table 1) or tolazoline [52]. Piperoxan, an α_2 -antagonist which potently blocks the action of clonidine [60–62], is not selective between both I₁- and α_2 -adrenergic receptor binding sites (Table 1).

Yohimbine and its diastereoisomers rauwolscine and corynanthine have been widely used to characterize α_2 -adrenergic receptors. Yohimbine and rauwolscine are equally potent at α_2 -adrenergic receptors, while corynanthine is much weaker [63]. Rauwolscine is equally potent at serotonin receptors as at α_2 -adrenergic receptors, and yohimbine is only moderately selective for α_2 relative to serotonin receptors [57–59]. Yohimbine is only fivefold more potent than corynanthine in reversing the hypotensive effect of clonidine, whereas yohimbine is 100-fold more potent than corynanthine in blocking clonidine's sedative action [63]. In α_2 -adrenergic binding studies, yohimbine is also 100-fold more potent than corynanthine [12,63]. The

agreement between relative α_2 -adrenergic potencies and blockade of sedative actions, contrasted with the lack of agreement between α_2 -adrenergic potencies and blockade of hypotensive actions, implies a role for a receptor with equal affinities for yohimbine and corynanthine, such as the I_1 -imidazoline receptor [12], in hypotension but not sedation. Consistent with this notion is a separate study showing that yohimbine and corynanthine were equally effective in blocking the antihypertensive action of clonidine in renal hypertensive rats [64]. Moreover, yohimbine antagonizes the hypotensive effect of clonidine in a biphasic manner, suggesting that more than one receptor is involved [65]. In rabbits, doses of yohimbine up to 3 mg/kg did not block the hypotensive response to clonidine [66]. Interestingly, the vasodepressor action of clonidine is not inhibited by several α_2 -antagonists which do not bind to I_1 -sites, such as ergot alkaloids [61,62] or phenoxybenzamine [61,67]. All of these results are at least compatible with a non-adrenergic contribution to central antihypertensive efficacy.

A second weakness of studies showing reversal of the action of clonidine and other central antihypertensive agents by α_2 -antagonists is that the antagonists were administered systemically [68]. Systemic administration of α -adrenergic antagonists greatly complicates the interpretation of results. Blockade of systemic α -adrenergic receptors, even if partial, will decrease the importance of the sympathetic nervous system in maintaining blood pressure. Typically, blood pressure may be maintained during α -adrenergic blockade by increased production of pressor hormones. Following blockade of vascular α_1 - or α_2 -adrenergic receptors, centrally mediated sympathoinhibition may lose effectiveness. Thus, the specific α_1 -adrenergic antagonist prazosin (1 mg/kg) attenuates the hypotensive response to clonidine nearly as effectively as yohimbine (1 mg/kg) [64,69]. This does not mean that clonidine's action is mediated by α_1 -adrenergic receptors, but rather that the antagonist has interfered with its mechanism of action downstream of the receptor. Similarly, the β -adrenergic antagonist propranolol can prevent or reverse the vasodepressor action of clonidine in animals or in human patients [70]. These considerations apply to studies where high doses (>1 mg/kg) of intravenous α_2 -adrenergic antagonists such as SK&F86466 [68,71] or yohimbine [72] were given prior to an I_1/α_2 agonist, also given intravenously. Blood pressure fell abruptly after intravenous SK&F86466 at 3 mg/kg (unpublished) and remained decreased at the time the agonist was administered [68,71,72]. In each of these studies, the absolute level of blood pressure is never given, only the fall elicited by the agonist. Of course, after treatment with an α_2 -adrenergic antagonist, the fall is from a lower baseline. For example, treatment of rabbits with SK&F86466 (3 mg/kg plus infusion of 1 mg/kg/h) lowered mean pressure 13% while increasing renal sympathetic nerve activity by nearly 50% [71]. Thus, the physiological state of the animal was dramatically altered by high doses of intravenous α_2 -adrenergic antagonists.

A relatively unambiguous example of data that contradict the I_1 -imidazoline receptor hypothesis is the effect of direct injection of the α_2 -adrenergic/5HT_{1A} antagonist yohimbine into the cisterna magna of the brainstem in the rabbit [72]. Pretreatment of the brainstem with yohimbine had little effect on its own, but appeared to prevent the hypotensive response to moxonidine. Unfortunately, there was also very little hypotensive effect of moxonidine in the control group. Only the highest dose of moxonidine (100 μ g/kg) decreased mean arterial blood pressure significantly, and this response was very small in magnitude (less than 6 mmHg). Thus, the authors' claim that brainstem α_2 -blockade with yohimbine abolished a 6 mmHg vasodepressor response is not convincing. The absence of an appreciable fall in blood pressure in response to moxonidine in the rabbits in this study probably results from a species difference in the ability of moxonidine to cross the blood-brain barrier, because rabbits are highly responsive to moxonidine delivered into the cisterna magna [73]. Importantly, plasma catecholamines were decreased by moxonidine, despite the absence of a clear vasodepressor effect, and the plasma catecholamine response was not attenuated by brainstem α_2 -adrenergic blockade with yohimbine [72]. In fact, yohimbine tended to potentiate the effect of moxonidine.

These results leave unsettled the relative contributions of I₁-imidazoline and α_2 -adrenergic receptors in the vasodepressor response to moxonidine in different species.

There are other pitfalls in neuropharmacological studies of the central vasodepressor response to imidazolines. The most clear-cut errors are those that result from false assumptions regarding the specificity of particular drugs, especially antagonists. For example, several studies have reached incorrect conclusions based on the assumption that methoxydazoxan (RX821002) is specific for α_2 -adrenergic receptors and does not interact with imidazoline sites. The source of this assumption appears to be a report showing that methoxydazoxan does not bind to the mitochondrial I₂-subtype of imidazoline [74]. Methoxydazoxan actually has moderate affinity for I₁-imidazoline receptors in the RVLM, similar to its parent compound, idazoxan (Table 1). Furthermore, in bovine adrenal chromaffin cells, methoxydazoxan again shows moderate affinity for I₁-imidazoline sites ($K_i = 226 \pm 73$ nM from analysis of four triplicate experiments each using 6–8 concentrations of methoxydazoxan with the LIGAND program [75]). The Charlottesville group found that methoxydazoxan increases blood pressure when microinjected into the RVLM [76], whereas under the same circumstances the selective α_2 -adrenergic antagonist rauwolscine lowers blood pressure [77]. Oddly, this group concluded that α_2 -adrenergic receptors in RVLM had a tonic vasodepressor influence [76], despite opposing data from their own laboratory [77]. Overall, the data reported to date show that only those α_2 -antagonists which are active at I₁-imidazoline receptors, such as efaroan and methoxydazoxan, elicit a small increase in blood pressure within the RVLM [15,76]. Conversely, α_2 -antagonists with low affinity at I₁-imidazoline receptors, such as SK&F86466, rauwolscine, and others actually tend to decrease blood pressure [29,77,78].

In a separate series of studies, an Australian group has used methoxydazoxan as a putative selective α_2 -adrenergic antagonist in contrast to efaroan [43,79]. They found that efaroan was much more potent in blocking the I₁-imidazoline agonists rilmenidine and moxonidine than was methoxydazoxan, whereas efaroan was less potent than methoxydazoxan for blocking α -methyl dopa, a pure α_2 -adrenergic agonist. These authors reached the correct conclusion, thanks to efaroan's greater I₁-receptor selectivity relative to methoxydazoxan, but the relative role of α_2 -adrenergic receptors was overestimated because of the incorrect assumption that methoxydazoxan does not interact with I₁-imidazoline receptors. This assumption has also led several radioligand binding studies to incorrect conclusions. Studies in dog kidney and in rat kidney concluded that I₁-imidazoline receptors were absent, solely on the basis of the ability of 10 μ M methoxydazoxan to inhibit specific binding completely [80, 81] or to block functional drug responses [82]. In the rat kidney, however, up to 34% of specific [³H]-p-NH₂-clonidine binding could not be inhibited by 1.0 μ M epinephrine [81], consistent with the presence of a subpopulation of I₁-imidazoline receptors. The expression of I₁-imidazoline receptors in dog kidney cannot be ruled out, particularly since these sites are known to be present in dog prostate [39].

In another recent study, mice with a mutant inactive form of the α_{2a} -adrenergic receptor were studied [24]. When the mutant mice were injected intra-arterially with the selective α_2 -adrenergic agonists D-medetomidine or bromonidine, they failed to show the fall in blood pressure seen in normal mice. This study demonstrates the role of the α_{2a} -subtype in the response to specific α_2 -adrenergic agonists. However, the authors concluded that their study ruled out any functional role of I₁-imidazoline receptors in the mouse, because they incorrectly assumed that because D-medetomidine or bromonidine share an imidazoline chemical structure, they must activate I₁-imidazoline receptors. As shown in Table 1, medetomidine has almost no affinity for I₁-imidazoline receptors. Similarly, bromonidine is nearly 100-fold selective for α_{2a} -adrenergic receptors relative to I₁-imidazoline receptors [52]. Thus, the question remains open whether mutant mice lacking functional α_{2a} -adrenergic receptors still retain I₁-imidazoline receptors in the RVLM which participate in blood pressure control.

In an attempt to overcome some of the pitfalls discussed above, we have examined the specificity of the antihypertensive action of moxonidine in SHR chronically treated with moxonidine via a subcutaneous osmotic minipump. After 28 days of moxonidine treatment (8 mg/kg daily), SHR were anesthetized with urethane (1.0 g/kg) and the carotid artery was cannulated. Chronic moxonidine completely normalized hypertension in these rats [treated ($n = 4$), 125 ± 9 mmHg; control ($n = 3$), 174 ± 3 mmHg]. We tested the effects of repeated intra-arterial injections of the selective α_2 -antagonist rauwolscline followed by administration of the I_1/α_2 -antagonist efaroxan. As shown in Table 1, rauwolscline has undetectable affinity for I_1 -imidazoline receptors but high α_2 -affinity. According to the I_1 -imidazoline receptor hypothesis, rauwolscline should be less effective in blocking the ongoing antihypertensive action of moxonidine than efaroxan. In contrast, the ' α_2 -receptor only' hypothesis predicts that rauwolscline should potently and completely reverse the effect of moxonidine and that the further addition of efaroxan, a second α_2 -adrenergic antagonist, should have no additional effect. The baseline mean arterial pressure was only 105 ± 4 mmHg (averaged across the last four 2 min readings), showing that chronic treatment with moxonidine (8 mg/kg daily by osmotic mini-pump) normalized blood pressure (Fig. 5). At intervals of 10 min, the rat was injected intra-arterially with cumulative doses of the α_2 -antagonist rauwolscline (a total of 0.1, then 0.3 and finally 0.5 mg/kg). Contrary to the α_2 -adrenergic hypothesis, rauwolscline further decreased blood pressure acutely. This might be due to blockade of the peripheral vasoconstrictor action of moxonidine mediated by vascular α_2 -adrenergic receptors. After a cumulative dose of 0.5 mg/kg rauwolscline, blood pressure did not differ from the baseline. We then delivered 0.2 mg/kg of the I_1/α_2 -antagonist efaroxan. In further contrast to the outcome predicted by the α_2 -adrenergic hypothesis, efaroxan rapidly and completely reversed the antihypertensive action of moxonidine. These data are consistent with the hypothesis that, with long-term systemic treatment, moxonidine acts mainly as an I_1 -imidazoline agonist. Administration of rauwolscline and efaroxan increased blood pressure only slightly (<20 mmHg) in four control SHR not implanted with osmotic minipumps. Thus, the effects shown in Fig. 5 do not reflect the direct effect of the antagonists on blood pressure. One limitation of this study is rauwolscline's high affinity for serotonin receptors [57–59], which may influence its central cardiovascular actions.

Relative potencies of moxonidine and clonidine in a test of sedative action

Clinical trials comparing moxonidine and clonidine have shown reduced sedative actions of the former agent, in a crossover trial [83] and in acute studies [84]. Moreover, moxonidine treatment may actually lead to an improvement in performance on a driving simulator [85], whereas patients receiving clonidine may be warned by pharmacists about operating heavy equipment owing to sedation. However, direct laboratory investigations of the sedative actions of moxonidine have thus far been lacking. In mice, clonidine is 21-fold more potent than moxonidine in reducing exploratory activity [86]. Unfortunately, the relative potency of clonidine and moxonidine in lowering blood pressure in mice is unknown. Rupp and colleagues continuously monitored both spontaneous locomotion and blood pressure by telemetry during oral dosing with moxonidine or clonidine in SHR [87]. They found that moxonidine at a dose of 8 mg/kg daily reduced locomotor activity by 10 arbitrary units on a scale of 0–300, and clonidine had a comparable effect at a 30-fold lower dose (0.3 mg/kg daily). Thus, clonidine was 27-fold more potent in reducing locomotion than moxonidine. However, clonidine was also 10-fold more potent than moxonidine in lowering diastolic blood pressure, implying that moxonidine was only threefold less potent than clonidine in reducing spontaneous locomotor activity at equieffective doses. The 10-fold lower antihypertensive effectiveness of moxonidine relative to clonidine after oral administration in SHR [87] is in close agreement with previous results with oral or subcutaneous routes of administration [86]. Because clonidine and moxonidine are nearly equally effective in correcting hypertension in SHR after direct microinjection into the RVLM [88] or following infusion into the cisterna magna [53], the

most likely explanation for the reduced effectiveness of moxonidine in the rat is a lesser ability to penetrate the blood–brain barrier relative to clonidine. In contrast to hypertensive rats, for human hypertensives moxonidine and clonidine are very nearly equally potent after oral administration [11,83], implying a species difference in the ability of moxonidine to penetrate the blood–brain barrier. This species difference must be taken into account when evaluating studies of moxonidine in the rat.

Spontaneous locomotor activity is not a reliable indicator of sedation, because it is affected by many other behaviors. Moreover, SHR in particular show increased locomotor activity relative to other rat strains of both hypertensive and normotensive phenotypes [89]. Therefore, we sought to determine the relative potencies of moxonidine and clonidine on a reliable behavioral test specifically designed to evaluate sedatives, the potentiation of hexobarbital-induced loss of the righting reflex [90]. Normotensive Sprague–Dawley rats were given clonidine, moxonidine or vehicle by the oral route and then injected intraperitoneally with hexobarbital at a dose of 34 mg/kg. Only 15% of vehicle-treated rats ($n = 20$) showed a loss of righting reflex and were therefore judged to be sedated. Clonidine dose-dependently increased the proportion of rats that were sedated (Fig. 6), with a median effective dose (ED_{50}) of 0.31 $\mu\text{mol/kg}$. Moxonidine also induced sedation, but was 165-fold less potent than clonidine on a molar basis. Given that moxonidine is 10-fold less potent than clonidine in lowering blood pressure in rats, we conclude that at equally effective antihypertensive doses, moxonidine is 16-fold less potent than clonidine in inducing sedation. These data on moxonidine are in agreement with a study in mice concerning another I_1 -selective agonist, rilmenidine, which showed that hexobarbital sleeping time was not prolonged by doses of rilmenidine up to 36 $\mu\text{mol/kg}$ [91]. The data shown in Fig. 6 confirm the reduced sedative action of moxonidine relative to its vasodepressor action as shown in human clinical trials.

Impact of moxonidine therapy on insulin resistance in an animal model of metabolic syndrome X

Human hypertension usually does not occur in isolation, but rather appears as a component of interrelated chronic disorders and risk factors. Syndrome X, originally described in humans by Reaven [92], consists of insulin resistance as a primary defect associated with compensatory hyperinsulinemia, impaired glucose tolerance, dyslipidemia, and hypertension. Obesity, while not an absolutely essential attribute of the syndrome, contributes to insulin resistance and is a frequent accompaniment to the syndrome. Recently, we have characterized a potential animal model of human syndrome X, the obese spontaneously hypertensive rat (SHROB; Koletsy rat) [93–95]. The SHROB expresses genetic obesity, spontaneous hypertension, hyperinsulinemia and hyperlipoproteinemia (type IV), features which closely resemble those found in the human ‘syndrome X’. Increasingly, clinicians are recognizing the importance of attacking multiple facets of metabolic syndrome X, and not simply addressing hypertension in isolation. Accordingly, we have tested whether moxonidine can affect metabolic derangements commonly associated with human essential hypertension. We found that not only was blood pressure lowered, but kidney damage was greatly reduced, body weight and food intake moderated, glucose tolerance improved, hyperinsulinemia partially reversed, and elevations in both triglycerides and cholesterol were attenuated [94]. In the present study, we focused on the impact of moxonidine therapy on insulin resistance, the central abnormality in human metabolic syndrome X [92].

As previously reported, moxonidine treatment for 90 days at 8 mg/kg daily in the drinking water effectively controlled hypertension in both SHR and SHROB [94]. Moxonidine prevented weight gain in SHROB and induced gradual weight loss, particularly during the first month of treatment. In contrast, moxonidine did not affect weight gain in lean rats.

Fasting blood glucose was close to 60 mg/dl in all groups and was not affected by moxonidine treatment (Fig. 7a, b). Among control animals, blood glucose levels following oral challenge were higher in SHROB than in SHR at 60, 90, 120 and 180 min ($P < 0.05$, Newman–Keuls test). Moxonidine improved glucose tolerance in both lean SHR and in SHROB. In SHR, blood glucose levels were reduced at 120 and 180 min post-challenge. In SHROB, blood glucose levels were lower in the moxonidine treated group from 60 min onwards. Moxonidine treatment completely reversed abnormal glucose tolerance in SHROB.

Immunoreactive insulin levels were measured in the same blood samples using rat insulin standards. In control animals, fasting insulin levels were elevated by a factor of 31 in SHROB relative to SHR (note difference in scale between Fig. 7c, d). An oral glucose load induced a sustained increase in plasma insulin which was proportionally commensurate in SHR and SHROB (increases of 349 and 345%, respectively, at 180 min post-challenge). Moxonidine treatment increased fasting insulin levels in SHR ($P < 0.05$, Newman–Keuls test) and facilitated the response to a glucose load after 90, 120 and 180 min. In SHROB rats, in contrast, fasting insulin was reduced by a factor of three ($P < 0.05$, Newman–Keuls test), whereas post-challenge plasma insulin levels were not affected by moxonidine therapy.

The data in Fig. 7 were analyzed by area under the curve, and the results are shown in Fig. 8. In control animals, glucose area was increased in SHROB relative to their SHR littermates expressing a lean phenotype (Fig. 8a), implying reduced glucose tolerance in SHROB relative to SHR. Moxonidine treatment in SHR produced only a non-significant trend toward improved glucose tolerance. In contrast, chronic moxonidine therapy decreased glucose area by a factor of three in SHROB. Remarkably, among moxonidine-treated animals glucose area was significantly lower in SHROB than in SHR [$P < 0.05$, Newman–Keuls after two-way analysis of variance (ANOVA)]. The areas under the curve for insulin (Fig. 8b) are probably underestimated, particularly for untreated SHROB, as insulin levels had not returned to the baseline by the end of the 180 min tolerance test. Note that a log scale for the ordinate was required to allow comparison of data for SHR and SHROB. In untreated animals, insulin area was increased by a factor of 24 in SHROB relative to SHR. Moxonidine treatment increased insulin area in both SHR and SHROB ($P < 0.05$, Newman–Keuls after two-way ANOVA). In SHROB, insulin area was increased even though glucose-stimulated insulin levels were unchanged, owing to reduction in baseline insulin which caused an increase in the apparent net stimulation of insulin. Moxonidine treatment induced a 54% increase in insulin area (7440 ± 1480 versus 4830 ± 730 h $\times$ ng/ml, $P < 0.05$ by Newman–Keuls), although this effect is minimized in Fig. 8b by the use of a logarithmic scale. In treated animals, insulin area was elevated 18-fold in SHROB relative to SHR, demonstrating the persistence of profound hyperinsulinemia even after moxonidine therapy.

Thus, moxonidine treatment ameliorated insulin resistance in SHROB, a model of human syndrome X. Moxonidine's effects in this model may not be entirely the result of SNS inhibition alone. The prevention of body weight gain in the SHROB may have contributed to the improved glucose tolerance and reduced insulin resistance, although SHR showed no change in body weight yet showed increased apparent glucose disposal and an enhanced insulin response to a glucose load. The I_1 -imidazoline receptor is present in brainstem autonomic regions, including the hypothalamus [34], and the hypothalamus regulates SNS and para-sympathetic activity as well as insulin levels and feeding behavior. Conceivably, activation of hypothalamic I_1 -imidazoline receptors by moxonidine may contribute to its therapeutic actions. Also possible is a direct effect of moxonidine to facilitate insulin secretion from the pancreas. However, studies of isolated rat pancreatic islets *in vitro* show that high concentrations of moxonidine (>10 μ M) acutely inhibit insulin release after inactivation of α_2 -adrenergic receptors [96], opposite to the effect of chronic moxonidine *in vivo* as shown in Figs 7 and 8. Further studies are required to clarify these contradictory findings.

A limitation of the present study is that we did not test selective antagonists or compare moxonidine to a selective α_2 -adrenergic agonist to determine whether the effects of moxonidine on syndrome X are mediated by I₁-imidazoline or α_2 -adrenergic receptors. However, clonidine in contrast to moxonidine, induces hyperglycemia and impairs glucose tolerance in rats [97, 98] and in humans [99]. In contrast, the selective I₁-imidazoline agonist moxonidine has been found to lower plasma glucose levels in patients [100]. Moxonidine's greater selectivity for I₁-imidazoline receptors relative to clonidine may account for its differential effects on insulin resistance and glucose tolerance. We conclude that moxonidine has multiple beneficial effects in obese hypertensive syndromes, which may result from activation of I₁-imidazoline receptors.

Summary and conclusions

The development of the I₁-imidazoline receptor hypothesis from *in vitro* observations of a binding site to a target for therapeutic action in the clinic was reviewed, and new information was presented broadening the base of knowledge about this novel class of receptor. For a more comprehensive picture of imidazoline receptor research, the reader is referred to two excellent reviews that summarize recent advances in research on I₁-imidazoline receptors [101,102].

In this article, we have shown that tritiated imidazoline ligands label specific binding sites in the RVLM, the brainstem location responsible for the antihypertensive actions of imidazolines. Certain of the binding properties of I₁-imidazoline sites are reproducible between different laboratories and different radioligands (Fig. 1), even though distinct low-affinity imidazoline sites are also observed by some groups. The latter binding sites bear no relationship to antihypertensive efficacy (Fig. 4). Selective α_2 -adrenergic agonists in use as selective sedative/anesthetic/analgesic agents, such as medetomidine, do not bind to I₁-imidazoline sites (Fig. 2, Table 1). The I₁-imidazoline receptor is coupled to specific transmembrane signaling pathways leading to the generation of the second messengers diacylglycerol and arachidonic acid (Fig. 3). The antihypertensive effect of chronic moxonidine treatment cannot be reversed by selective α_2 -adrenergic blockade, but is promptly reversed by efaroxan, a selective I₁-imidazoline antagonist (Fig. 5). Moxonidine is less potent than clonidine in producing sedation by a factor of 165 (Fig. 6), which can only partially be explained by a reduced ability to cross the blood-brain barrier in the rat. Chronic moxonidine treatment improves glucose tolerance in genetically hypertensive rats, particularly those with obesity and hypertension (Figs 7 and 8). Insulin secretion is increased and insulin resistance is reduced, as shown by lowered fasting insulin levels after moxonidine treatment in obese hypertensive rats. Therefore, moxonidine may be an effective and well-tolerated treatment for hypertension, particularly for hypertension marked by insulin resistance, known as metabolic syndrome X.

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Fig. 1.

Radioligand binding experiments in bovine rostral ventrolateral medulla (RVLM) membranes demonstrating the presence of non-adrenergic binding sites specific for imidazolines. Inhibition of radioligand binding by increasing concentrations of clonidine (circles) or (–)-norepinephrine (squares) is shown. Each point represents the mean of three to seven separate experiments conducted in triplicate. Data are expressed as a percentage of total specific binding and replotted and reanalyzed from separate reports from laboratories in New York City [12] and Strasbourg [16] obtained using different radioligands: [^3H]-p-NH $_2$ -clonidine (PAC) [12] and [^3H]-clonidine [16]. Non-specific binding was defined in the presence of 10 and 100 μM phentolamine for [^3H]-PAC and [^3H]-clonidine, respectively. The bovine RVLM was used because of its large size and ready availability with a short post-mortem delay. Affinity values (K_i) are as follows: clonidine versus [^3H]-PAC, 17 ± 2 nM; clonidine versus [^3H]-clonidine, 17 ± 4 nM; norepinephrine versus [^3H]-PAC, 99 ± 29 nM for $62 \pm 2\%$ of sites; norepinephrine versus [^3H]-clonidine, 32 ± 9 nM for $69 \pm 3\%$ of sites. Data were analyzed and competition curves generated using the Prism program (GraphPAD, San Diego, California, USA). Note the close agreement between data from two separate labs [12,16] using different radioligands.

Fig. 2.

Radioligand binding experiments in bovine rostral ventrolateral medulla (RVLM) membranes demonstrating the selectivity of the anesthetic/analgesic agents detomidine and medetomidine for α_2 -adrenergic receptors relative to I_1 -imidazoline receptors. Inhibition of [3 H]-p-NH $_2$ -clonidine binding by increasing concentrations of detomidine (squares) or medetomidine (circles) is shown. Data represent the mean $\pm$ SE from four experiments conducted in triplicate, are expressed as a percentage of total specific binding, and are reported here for the first time. Non-specific binding was defined in the presence of 10 μ M phentolamine. Data were analyzed and competition curves generated using the Prism program (GraphPAD, San Diego, California, USA). Note that detomidine and medetomidine bind with high affinity only to about half the specific binding sites labeled by [3 H]-p-NH $_2$ -clonidine in the RVLM. The remaining half shows low affinity. The sites displaying low affinity for detomidine and medetomidine appear to be I_1 -imidazoline sites. Relative to Fig. 1, a greater proportion of [3 H]-p-NH $_2$ -clonidine binding sites in the RVLM are I_1 -imidazoline sites (49 versus 36%), probably because of optimization of assay conditions [26].

Fig. 3. Hypothetical model of signaling mechanisms coupled to I₁-imidazoline receptor (I₁R) activation. PC-PLC, phosphatidylcholine-selective phospholipase C; PC, phosphatidylcholine; Choline PO₄, choline phosphate; DAG, diacylglyceride; PKC, protein kinase C; PGE₂, prostaglandin E₂.

Fig. 4.

Relationship between vasodepressor potency of compounds upon direct microinjection into the rostral ventrolateral medulla (RVLM) of Sprague–Dawley rats and their potency in binding assays in RVLM membranes of (a) high-affinity I_1 -imidazoline receptors [52] or (b) low-affinity imidazoline sites [16,56]. Shown on the y-axis is the net fall in mean arterial pressure, relative to vehicle control, of a standardized microinjection of a single dose (1 nmol) into the RVLM of normotensive Sprague–Dawley rats under urethane anesthesia (1.0 g/kg) [10,29, 103]. Shown on the x-axis in (a) is the negative log of the binding affinity (pK_i) at high-affinity I_1 -imidazoline sites in bovine RVLM [52]. Shown on the x-axis in (b) is the pK_i at low-affinity imidazoline sites in post-mortem samples of human RVLM [16,56]. Note that vasodepressor potency correlates with relative ranking at high-affinity I_1 -imidazoline sites ($r = 0.82$) but not at low-affinity imidazoline sites ($r = -0.18$). Note also that the binding affinities in (b) are 1–3 log units lower than those in (a), consistent with the designation of the former as low-affinity sites.

Fig. 5.

Effect of increasing cumulative doses of the α_2 -antagonist rauwolscine and subsequent treatment with the I_1/α_2 -antagonist efroxan on mean arterial blood pressure in an spontaneously hypertensive rats (SHR) implanted with an osmotic minipump delivering moxonidine. Three SHR were treated with moxonidine at a dose of 8 mg/kg daily by implantation for 28 days with an osmotic minipump (Alzet) containing either moxonidine free base in 20% dimethylsulfoxide/80% 0.1 M acetic acid. On day 28, SHR were anesthetized with urethane (1 g/kg intraperitoneally) and the femoral artery was cannulated for direct measurement of arterial blood pressure and intra-arterial administration of antagonists. Mean arterial pressure was recorded every 2 min. Data are shown from a single animal, representative of the results from three rats. The dashed line indicates basal level of mean arterial pressure determined during a 10 min baseline period. Doses indicated in the figure are in mg/kg bodyweight. Administration of the α_2 -adrenergic antagonist rauwolscine at a dose of 0.1 mg/kg immediately lowered mean arterial pressure from 105 to 64 mmHg. By the time a cumulative dose of rauwolscine of 0.5 mg/kg had been administered, mean arterial pressure had returned to its starting level. At 30 min after the first dose of rauwolscine, the I_1 -imidazoline antagonist efroxan was administered at a dose of 0.2 mg/kg. Mean arterial pressure promptly rose to the level of vehicle-treated SHR. The failure of a potent α_2 -adrenergic antagonist to reverse the antihypertensive action of moxonidine, and the effectiveness of an I_1 -imidazoline antagonist support a role in the antihypertensive actions of moxonidine for I_1 -imidazoline receptors.

Fig. 6.

Dose-dependent sedative action of moxonidine relative to clonidine following oral administration in rats. Female Sprague–Dawley rats weighing 100–125 g were given increasing doses of clonidine, moxonidine or vehicle (1% Tylose, 0.2% Tween-80) by gavage at 10 ml/kg bodyweight. One hour later, hexobarbital (10% in 1.0 M NaOH) was administered intraperitoneally at a dose of 34 mg/kg. This dose of hexobarbital is known to be just below the threshold for sleep induction. Rats were tested for the loss of righting reflex during the first 1 min after hexobarbital administration. Rats which did not right themselves were counted as sedated. Ten rats were tested in each group, and each rat was tested only once. Data were analyzed by non-linear regression fitting to a logistic equation of variable slope using the Prism program (GraphPAD, San Diego, California, USA). The dose–response curves were also generated by the program and represent the best fit. On a molar basis, clonidine was 165-fold more potent than moxonidine in potentiating the sedative action of hexobarbital: median effective dose (ED₅₀) clonidine, $0.31 \pm 0.1 \mu\text{mol/kg}$; ED₅₀ moxonidine, $51 \pm 3 \mu\text{mol/kg}$. The slopes of the dose-response curves (n_H) did not differ between drugs: clonidine, 1.14 ± 0.53 ; moxonidine, 1.37 ± 0.15 .

Fig. 7.

Oral glucose tolerance tests in (a, c) spontaneously hypertensive rats (SHR) and (b, d) obese SHR (SHROB) with and without moxonidine treatment (8 mg/kg daily for 90 days). SHROB and SHR were treated with moxonidine at a dose of 8 mg/kg daily in their drinking water for 90 days. The concentration of moxonidine in each rat's water bottle was adjusted weekly according to changes in fluid consumption and body weight. Saccharin (0.1%) was added to the moxonidine solution to maintain palatability. For oral glucose tolerance testing, rats were fasted for 18 h and then given 12 g of glucose per kg body weight by gavage. Blood (0.7 ml) was obtained from a cut on the tail at the times shown and glucose was measured by colorimetric glucose oxidase assay (One-Touch, Lifescan, Milpitas, California, USA). A radioimmunoassay kit (Linco Research, St Charles, Missouri, USA) was used with rat insulin standard and antibodies directed against rat insulin. Assays were conducted in duplicate and the intra-assay coefficient of variation was less than 5%. All values are given as means $\pm$ SE obtained from seven animals in each group of SHR, and eight animals in each group of SHROB.

Fig. 8.

Area under the curve for glucose tolerance tests in lean spontaneously hypertensive rats (SHR) and obese SHR (SHROB) phenotypes with and without chronic moxonidine treatment for (a) glucose and (b) insulin. Glucose tolerance curves presented were analyzed for area using the trapezoidal method (Prism program, GraphPAD). Note that insulin areas under the curve are plotted on a logarithmic scale to facilitate comparisons between groups. Data were analyzed by using a two-way analysis of variance by phenotype and drug treatment and Newman–Keuls post-hoc analysis. *Significant effect of phenotype, with SHROB > SHR in all cases except glucose area under the curve after moxonidine treatment, where SHROB < SHR. †Significant effect of moxonidine treatment, with glucose area under the curve being significantly decreased by moxonidine in SHROB and insulin area under the curve increased by moxonidine in both SHROB and in SHR.

Table 1

Radioligand binding properties of representative compounds at I₁-imidazoline and α₂-adrenergic receptors in bovine rostral ventrolateral medulla membranes

	K _i (nM)		Selectivity ratio	Reference
	I ₁ -Imidazoline sites	α ₂ -Adrenergic sites		
Imidazoline antihypertensive agents				
Moxonidine	2.3±0.5	75±8	32.61	[54]
Rilmenidine	6.1±1.5	180±14	29.51	[54]
Clonidine	1.0±0.3	3.8±1.0	3.80	[54]
Lofexidine	1.9±0.7	6.9±0.7	3.63	[54]
Non-imidazoline antihypertensive agents				
Guanabenz	>10000	7.2±0.6	0.00072	[54]
Guanfacine	2500±190	2.3±0.7	0.00092	[54]
α-Methylnorepinephrine	89000±34000	73±16	0.00082	[29]
Sedatives/anesthetics/analgesics				
Detomidine	2400±900	4.1±1.6	0.00171	This report
-Medetomidine	14600±4500	2.7±0.9	0.00018	This report
Antagonists				
Efaroxan	0.15±0.06	5.6±1.4	37.33	[15]
Pieroxan	44±20	40±9	0.90909	This report
Idazoxan	186±17	3.6±0.85	0.01935	[29]
Methoxyidazoxan (RX821002)	400±85	2.1±0.8	0.00525	This report
SK&F86466	93000±150000	35±8.2	0.00038	[29]
Rauwolscine	>100000	5.6±3.6	0.00006	This report

Affinity constants (K_i) are ligand concentrations (nM) ± SE of the estimate obtained by using the LIGAND program [75] as previously described [54] or the Prism program (see Fig. 2). The Selectivity Ratio is the K_i at α₂-adrenergic receptors divided by the K_i at α₁-adrenergic receptors.