Imidazoline Receptors, Novel Agents and Therapeutic Potential

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Abstract: The initial realization that agents containing an imidazoline structure may interact with a distinct class of receptors, has led to a major class of cardiovascular agents, which now has the potential to enter a third generation. There is now general acceptance that there are three main imidazoline receptor classes, the I_1 imidazoline receptor which mediates the sympatho-inhibitory actions to lower blood pressure, the I_2 receptor which is an important allosteric binding site of monoamine oxidase and the I_3 receptor which regulates insulin secretion from pancreatic β cells. Thus all three represent important targets for cardiovascular research. Interestingly, an I_1- receptor candidate has been cloned (IRAS, imidazoline receptor antisera selected) which is a homologue of the mouse cell adhesion integrin binding protein Nischarin. There has been range of new agonists and antagonists with very high selectivity for I_1, I_2 and I_3 receptors developed. Three different endogenous ligands have been characterized including agmatine (decarboxylated arginine), a range of β-carbolines including harman and harmane, and more recently imidazoleacetic acid-ribotide. The imidazoline field has recently seen an enormous diversification with discoveries that I_1 and I_2 receptors also play a role in cell proliferation, regulation of body fat, neuroprotection, inflammation and some psychiatric disorders such as depression. This diversification has continued with the addition of effective agents with imidazoline affinity in the fields of cancer, pain and opioid addiction, stress, cell adhesion, epilepsy and appetite. The imidazoline field has maturated considerably with a range of highly selective leader molecules, candidate receptors and endogenous ligands. We are therefore only at the threshold of an exciting new era as we begin to understand the diverse and complex nature of their function.

Key Words: Imidazoline, clonidine, rilmenidine, moxonidine, cardiovascular regulation, centrally acting drugs, antihypertensive agents.

INTRODUCTION

The field of imidazoline receptor research began in the 1960's with the discovery of the hypotensive properties of an imidazoline derivative clonidine, which was originally developed to be used as a nasal decongestant [1,2]. The hypotensive action of clonidine was soon shown to be mediated by a central inhibition of sympathetic tone [3], which was initially thought to involve α_2-adrenoceptors [4]. The major site of action was found to be the rostral ventrolateral medulla (RVLM) [5]. However, it became evident that the α_2-adrenoceptor mechanism of action for clonidine could not completely explain its hypotensive effect, and an alternative hypothesis was created involving a specific receptor for the imidazoline structure. Karppanen and colleagues in 1977 suggested that clonidine might act *via* a non-adrenergic site that recognised imidazole-containing ligands with an affinity for the histamine receptor [6]. Interestingly, they noted that imidazole-acetic acid, a metabolite of histamine, exerts a strong hypotensive action when injected ICV in anaesthetized rats [7] which foreshadowed, by 27 years, the very recent work by Prell and colleagues who found that imidazole-acetic-acid ribotide is an important endogenous ligand for imidazoline receptors in the brain [8]. One of the key steps in the formation of the imidazoline receptor hypothesis was the observations of Ruffolo and colleagues who showed a lack of cross desensitization of rat vas deferens by phenethylamines and imidazoline agonists which indicates an action at quite separate sites [9]. Together, these reports suggested the potential existence of non-adrenergic imidazoline receptors and Bousquet and colleagues, provided evidence that the antihypertensive action of clonidine and other imidazoline-related compounds was due to their interaction, in the RVLM, with imidazoline receptors rather than with α_2-adrenoceptors [10,11]. They found a positive correlation between the hypotensive potency of imidazoline compounds and their affinity for imidazoline receptors but not for α_2-adrenoceptors [12,13]. This concept, however, has not been universally accepted due to difficulties in separating functional effects of an interaction with α_2-adrenoceptors from those of imidazoline receptors [14]. Consequently, the search for imidazoline selective agents, the identity of the imidazoline receptors and their the endogenous ligands have been the driving force in the field of imidazoline research for the last three decades. This has resulted in a rapid expansion of imidazoline receptor research particularly in the 1970's and 1980's. At this time there was an exponential growth in publications (Fig. **1**) reaching a peak rate in 1988. The second-generation agents, such as rilmenidine and moxonidine, were developed in the 1980's and these have been in clinical use with good success due to their lesser

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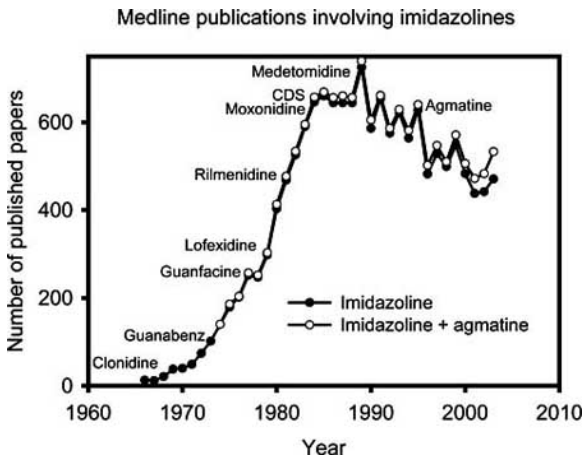


Fig. (1). Total number of publications per year for the keyword imidazoline, clonidine, moxonidine and rilmenidine (solid symbols) and also those plus agmatine (open symbol). However, it should be noted that early agmatine publications were focused on plants and bacteria. Along the line shows the sequence and approximate year (right hand side) in which various agents and substances were first described.

side effects typically associated with the α_2-adrenoceptor agonists.

In the last 10 years, although the rate of publications has fallen somewhat from the peak, a number of milestones have been achieved. In particular, highly selective novel imidazoline agents have been developed with virtually no α_2-adrenoceptor activity [15], and three distinct classes of imidazoline binding sites have been characterized. The I_1 receptor has been defined by the high affinity clonidine binding and is responsible for the hypotensive properties of the drug. Recently, a signal transduction system has been described for the I_1 receptor, and a candidate protein has been cloned called imidazoline receptor antisera selected (IRAS) which satisfies a number of the criteria of the I_1 receptor [16,17]. The I_2 binding site was originally described as imidazoline-guanidinium receptive site (IGRS) and character-ized by idazoxan binding [18]. This site has been identified as an allosteric binding site on monoamine oxidase (MAO) but recent evidence suggests that a number of binding sites may exist on other non-MAO oxidative enzymes [19]. The I_3 binding site has been suggested as inducing insulin secretion from pancreatic β cells in a manner, which is not typical of I_1 or I_2 receptor related phenomenon [14,20].

One of the most interesting aspects of the research has been the quest for the endogenous ligand for imidazoline receptors. The original isolation of a clonidine displacing substance (CDS) occurred in 1984 [21], but only in 2004 imidazole-acetic acid ribotide has been identified as a very likely candidate for the original CDS possessing almost all of its characteristics [8]. In the meantime, agmatine was recognized as a distinct endogenous ligands for imidazoline sites in 1994 [22] and more recently harmane [23] and other β-carbolines [24]. The structure of these various endogenous substances as well as the various ligands, agonists and antagonists is quite diverse (Fig. **2**).

Thus there has been considerable progress in imidazoline research in the past three decades and while it is important to acknowledge this, it is also important to look to the future. What are the remaining challenges and where is the field heading? It is clear that imidazoline compounds and receptors are becoming recognized in increasingly diverse areas. This was highlighted at the 4th International symposium on agmatine and imidazoline systems held in 2003. There were relatively few presentations concerned with the cardiovascular system, the area that provided the origins of the imidazoline receptor concept. By contrast, imidazoline receptors and binding sites have now been implicated in such diverse fields as cancer, pain, opioid addiction, depression, stress, cell adhesion and proliferation, epilepsy, appetite control and body fat composition, neuroprotection and inflammation. In the present review we provide a brief update to the field of imidazoline research, highlighting some of these newer areas and more recent exciting discoveries.

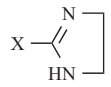
I_1-IMIDAZOLINE RECEPTORS

I_1-Receptors and Central Control of Blood Pressure

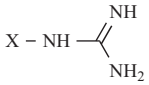
From the first suggestion of the existence of imidazoline receptors, there has been the continuing debate about their contribution to the antihypertensive actions of clonidine-like agents [14]. Early evidence showed that the hypotension produced by injection of imidazoline compounds into the RVLM was more related to their imidazoline structure than their affinity for α_2-adrenoceptors [11,13]. Potent α_2-adrenoceptor antagonists have been available with low affinity for imidazoline receptors, such as yohimbine, 2-methoxyidazoxan (2MI) and SK&F 86466 [25,26]. However, until recently, equivalent selective imidazoline antagonists have not been available. Unfortunately, the most frequently used imidazoline antagonists, idazoxan and efaroxan, are also potent α_2-adrenoceptor antagonists. This has made the task of distinguishing between imidazoline receptor and α_2-adrenoceptor mechanisms difficult, requiring a combination of agonists and antagonists with differing affinities for each receptor.

Much of the controversy has centered on clonidine, which has a relatively modest selectivity for imidazoline receptors over α_2-adrenoceptors (Table **1**). The hypotension induced by clonidine can be prevented by the α_2-adrenoceptor antagonist yohimbine, an α_2-antagonist that binds poorly to imidazoline receptors, in conscious spontaneously hypertensive rats (SHR) but not in normotensive rats [12]. These findings suggest that the contribution of imidazoline or α_2-adrenoceptors to the hypotensive action of clonidine may depend on the animal model used and perhaps on the relative proportion of these receptors in the RVLM, which is the main site of hypotensive action [10,13,27]. With more selective agonists, such as rilmenidine and moxonidine (30 fold selectivity for imidazoline receptors, Table **1**), the importance of imidazoline receptors for the hypotension is more readily seen. Mayorov and colleagues reported that hypotensive action of rilmenidine, given into the cisterna

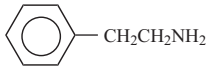
Imidazoline agonists



Guanidines

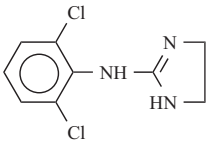


Phenylethylamines

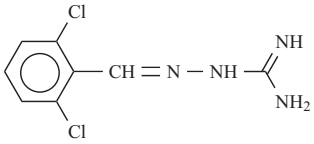


Amino-imidazolines

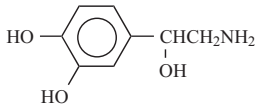
Clonidine



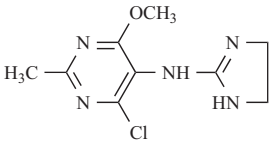
Guanabenz



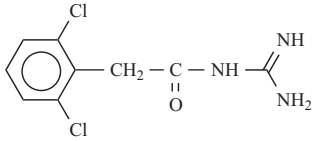
Noradrenaline



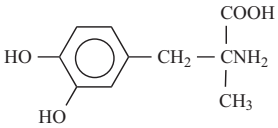
Moxonidine



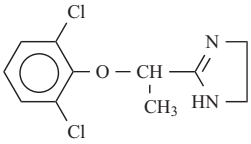
Guanfacine



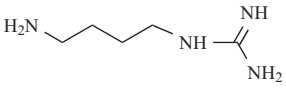
α-Methyldopa



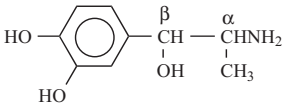
Imidazolines Lofexidine



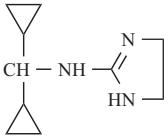
Agmatine



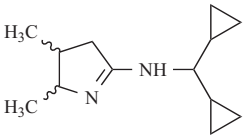
α-Methylnoradrenaline

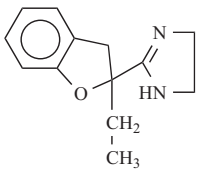
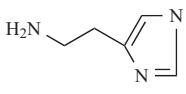
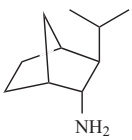


Oxazolines Rilmenidine



Pyrrolamines LPN509



Imidazoline antagonists	**Imidazoles**	**I_1 ligands**
Efaroxan 	Histamine 	**Cycloheptane analogue AGN 192403** 

(Fig. 2. Contd….)

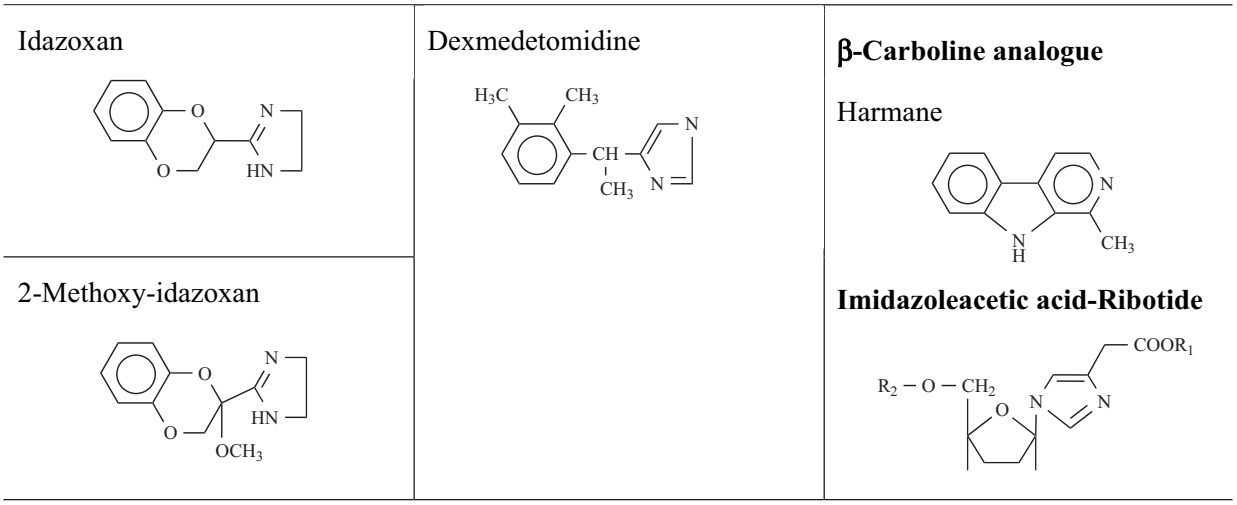


Fig. (2). Chemical structure of phenylethylamines, imidazolines and related ligands, X denotes different substituents.

magna, was attenuated by idazoxan but not equimolar to 10-fold higher doses of yohimbine in conscious barodenervated rats [28]. Haxiu and colleagues found that microinjection of a highly selective α_2-adrenoceptor antagonist SKF 86466 into the RVLM of anesthetized rats did not affect the hypotension induced by intravenous moxonidine. By contrast, the moxonidine-induced hypotension was blocked by the I_1 antagonist efaroxan [29].

The approach taken by our own studies has been to calibrate the different antagonists for their antagonist ability against a specific α_2-adrenoceptor agonist α-methyldopa. We found that rilmenidine injected into the fourth ventricle of conscious rabbits was reversed by idazoxan but not by 2MI [30]. By contrast clonidine was equally reversed by these two antagonists suggesting that in conscious rabbits, like SHR, clonidine is acting primarily at α_2-adrenoceptors [30]. This finding was confirmed with efaroxan, which was more effective than 2MI (both given into the fourth ventricle) at reversing the effects of rilmenidine and moxonidine (given intravenously or into the fourth ventricle) while 2MI was more effective than efaroxan at reversing clonidine [31,32]. These studies suggested that I_1 receptor was the target for rilmenidine and moxonidine but that clonidine acted mainly through α_2-adrenoceptors. Others have confirmed this for rilmenidine and moxonidine [33] using the same approach and also for the sympatho-inhibition produced by α_2-adrenoceptor agonist guanabenz [33]. We have also shown that with chronic administration, the dominance of the α_2-adrenoceptors mechanism diminishes and clonidine then lowers blood pressure mainly through its imidazoline actions [34]. This is consistent with the diminution, with chronic clonidine treatment, of sedation and other side effects that have been attributed to α_2-adrenoceptors [35]. Thus, by careful comparative pharmacology, we have shown that second-generation imidazoline agents, rilmenidine and moxonidine, produce their hypotension mainly through imidazoline receptors.

The objection to the imidazoline hypothesis has come from *in vivo* pharmacological studies using antagonists at α_2-adrenoceptor to block the actions of rilmenidine and moxonidine [36-38]. Also, electrophysiological studies of Guyenet and colleagues have demonstrated that α_2-adrenoceptors are involved in the inhibitory action of clonidine on vasomotor cells in the RVLM [39-41]. Furthermore, it has been suggested that the lack of hypotensive effect of centrally acting agents in mice with mutated and functionally less active α_{2A}-adrenoceptors [42] is evidence against the imidazoline hypothesis. It should be pointed out that these studies have only shown the importance of central α_2-adrenoceptors in the antihypertensive action of clonidine like drugs but have not ruled out the involvement of imidazoline receptors at all.

We proposed in 1994 that imidazoline receptors may be "in series" with α_2-adrenoceptors and that stimulation of the imidazoline receptor leads to activation of the α_2-adrenoceptor [43]. This explanation was based on observations that at higher doses the selective α_2-adrenoceptor antagonist 2MI could reverse the actions of intracisternally given rilmenidine [43]. However, these doses of 2MI were much higher than those required to block alpha-methyldopa. More recently, this interaction between α_2-adrenoceptors and I_1 receptors has been shown to occur within the RVLM itself [44] which contains both imidazoline receptors and α_2-adrenoceptors [45]. The hypotension produced by rilmenidine micro-injected into the RVLM was reversed by both idazoxan and 2MI [44]. By contrast, injections into the RVLM of α-methylnoradrenaline produced hypotension, which was dose-dependently reversed by microinjection of 2MI, but idazoxan administered in doses up to 40 nmol was without effect and therefore does not appear to block α_2-adrenoceptors. Since idazoxan reversed rilmenidine's effect, but not that of α-methylnoradrenaline, it suggests that rilmenidine acts on imidazoline receptors to produce sympatho-inhibition. The reversal by 2MI of rilmenidine's effect suggests that α_2-

Table 1. Selectivity of Drugs for α_{2A}-Adrenergic and I_1-Imidazoline Binding Sites

	α_{2A} site Ki (nM)	I_1 site Ki (nM)	Selectivity ratio (α_{2A}/I_1)	Species
Imidazoline-related agonists				
Clonidine	3.8	1.0	3.8	Cow[226]
Lofexidine	6.9	1.9	3.6	Cow [226]
Moxonidine	75	2.3	32.6	Cow [226]
Rilmenidine	180	6.1	29.5	Cow [226]
d,l-Medetomidine	2.7	14600	0.0002	Cow [26]
Specific I_1 ligands				
AGN 192403	> 20000	42	476	Cow[a] [135]
Benazoline	18000	9	2000	Rat [140]
BU98008	28000	82	342	Rat [142]
LPN509	>10000	538	>18	Cow [227]
LPN911	>10000	0.2	>50000	PC12 cells [228]
S23515	>10000	6.4	>1600	Cow [15]
S23757	>10000	5.3	>1900	Cow [15]
Guanidine agonists				
Guanabenz	7.2	> 10000	0.0007	Cow [226]
Guanfacine	2.3	2500	0.0009	Cow [226]
Agmatine	4000 46980	700 33.4	5.7 1407	Cow [22] Man[b] [229]
Phenylethylamine agonist				
α-Methylnoradrenaline	73	89000	0.0008	Cow [13]
Imidazoline antagonists				
Efaroxan	5.6	0.15	37.3	Cow [230]
Idazoxan	3.6 4.4	186 23.6	0.02 0.2	Cow [13] Man [231]
S23757	>10000	5.3	1890	Cow [15]
2-Methoxyidazoxan	2.1 4.8	400 7200 0	0.005 0.0007	Cow [26] Man[c] [232]
α_2-adrenergic antagonists (alkaloid or non-Imidazoline)				
SK&F 86466	35	93000	0.0004	Cow [13]
Rauwolscine	5.6	> 100000	0.00006	Cow [26]
Yohimbine	22 179	21810 3200	0.001 0.06	Rabbit[d] [233] Cow[e] [234]

Data represent the affinity constant (Ki) of drugs, in ligand concentration (nM) ± standard error, for α_{2A}-adrenergic (α_{2A}) and I_1-imidazoline (I_1) sites in the RVLM of the same species, unless otherwise stated. Adapted from Chan [225].

[a] Affinity for I_1 site in bovine RVLM and for α_{2A} site in Chinese hamster ovary (CHO) cells transfected with human α_{2A}-adrenoceptor cDNA (cell line AUA-C_{10}).

[b] Affinity for I_1 site in human platelet and for α_{2A} site in transfected CHO cells.

[c] Affinity for I_1 and α_{2A} binding sites in human frontal cortex.

[d] Affinity for I_1 and α_{2A} binding sites in rabbit frontal cortex.

[e] Affinity for I_1 site in bovine RVLM and for α_{2A} site in bovine frontal cortex.

adrenoceptors are activated, but most likely, as a consequence of an interaction with imidazoline receptors in the RVLM. Studies by Bousquet and colleagues have also demonstrated this interaction between imidazoline receptors and α_2-adrenoceptor [46]. Furthermore they have suggested that there is a marked synergy between the imidazoline receptor and α_2-adrenoceptors with studies that found that the effects of a selective imidazoline agent LPN 509 were potentiated by the activation of α_2-adrenoceptors using α-methylnoradrenaline [46,47]. They further contend that there would be a lack of synergy in the D79N mouse (lacking a functional α_2-adrenoceptor) which they demonstrate with the selective imidazoline agent LPN 509 [46]. This may also explain the findings with the α_{2A}-adrenoceptor mutated mouse where, without a functional α_2-adrenoceptor, the hypotensive action of imidazoline agents was markedly attenuated [42,48]. Thus, much of the difficulty in separating the contribution of these two receptors is probably due to their being part of the same autonomic pathway (Fig. **3**).

Electrophysiological and morphological studies have shown that α_2-adrenoceptors in the RVLM are located on presympathetic cell bodies as well as presynaptically on non-adrenergic terminals [49,50]. The effect of 6-OHDA to inhibit the moxonidine-induced hypotension and not that elicited by clonidine [51] suggests that imidazoline receptors may be on or upstream from presynaptic sympatho-inhibitory noradrenergic terminals. Thus inactivation of I_1 receptors by antagonists [44] or by intracisternally injected neurotoxins [51] inhibits the hypotensive effects of the I_1 selective agents but inactivation of α_2-adrenoceptors inhibits the both types of agents (Fig. **3**). These studies provide some of the clearest separation of the imidazoline actions from those of α_2-adrenoceptors, as there can be no argument about the selectivity of antagonists. We have recently extended these findings using a localized destruction of noradrenergic terminals in the RVLM by 6-OHDA [52,53]. We found that moxonidine responses were markedly attenuated while those of clonidine remained unchanged. Thus, we can now suggest that the action of moxonidine on the noradrenergic mechanisms controlling blood pressure occurs at the level of the RVLM. In further studies we have shown that this is accompanied by a specific reduction in the levels of a 43KDA protein recognized by a imidazoline receptor protein antibody [52,53].

There has been an alternative suggestion that the responses of various imidazoline agonists and antagonists in

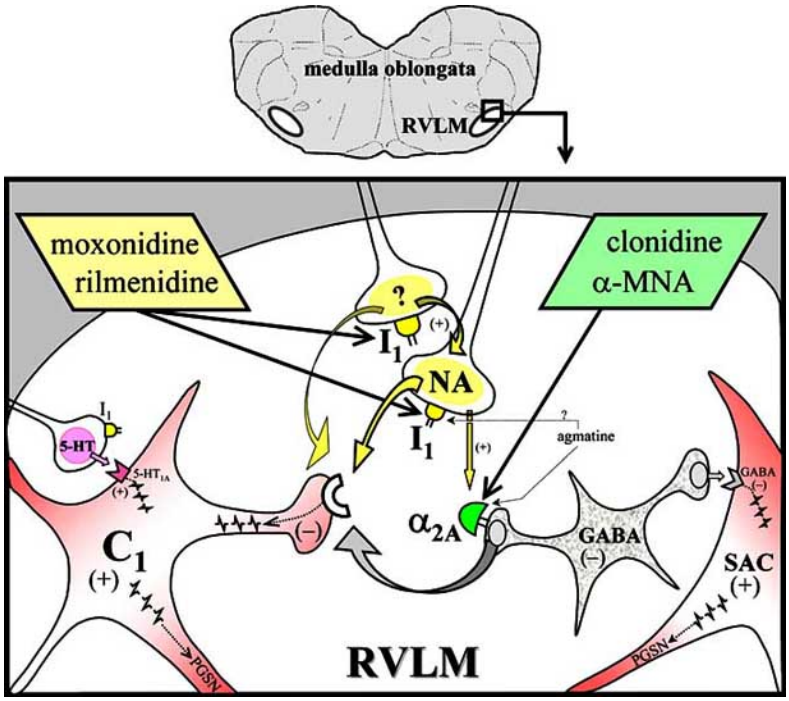


Fig. (3). Schema showing the anatomical connections of the I_1-receptors (I_1;) and α_{2A}-adrenoceptors (α_{2A};) in the rostral ventrolateral medulla (RVLM) of the rabbit. The C_1 adrenergic cell provides excitatory signals to the pre-ganglionic sympathetic neurons (PGSN) in the intermediolateral cell column of the spinal cord to alter sympathetic discharges to the peripheral tissues. Its activity can be inhibited by neurotransmitters that are released from the activation of I_1-receptors located on the noradrenergic (NA) and unknown (?) cell terminals, or from the stimulation of terminal α_{2A}-adrenoceptors located on the interneuron containing γ-aminobutyric acid (GABA). This interneuron also synapses onto the non-adrenergic spontaneously active cell (SAC). The serotonergic (5-HT) innervation may enhance the excitatory signals from the C_1 cell. The agonistic interactions of moxonidine and rilmenidine with I_1-receptors, clonidine and α-methylnoradrenaline (α-MNA) with α_{2A}-adrenoceptors, and agmatine with both groups of receptors are indicated. (+) Excitatory signals or cells; (–) inhibitory signals or cells; 5-HT_{1A} receptor; inhibitory receptor on C_1 cell; GABA receptor; block arrows (curved or straight) represent the neurotransmitter release from cell terminals. Figure adapted from Chan [225].

the RVLM may simply be due to differences in the degree of intrinsic activity and occupancy of α_2-adrenoceptors [14]. However, this hypothesis is not based on experimental findings. In fact, the dose-response curves for agonists acting through α_2-adrenoceptors are parallel to those for rilmenidine and moxonidine, which have been suggested by the above studies to act through imidazoline receptors [31,32]. Furthermore, the antagonist dose-response curves to idazoxan, efaroxan and 2MI reversing the actions of α-methyldopa and rilmenidine are all parallel [31,32,43]. This would not be the case if differential occupancy of the α_2-adrenoceptor were occurring in these studies.

I_1-Imidazoline Receptors in Essential Hypertension

Growing evidence indicates that neural control of the circulation *via* the sympathetic nervous system is altered in the early phase of hypertension and that this may be a contributory factor to the disease [54,55]. Sympathetic activity is not generally altered but is elevated to specific organs, such as the heart and kidney, in young borderline hypertensive patients [56]. Furthermore, it is now well established that in hypertensive humans and animals there are diminished baroreceptor reflexes [57-59]. Much has been written about the use of clonidine and also the second generation agents rilmenidine and moxonidine in treating hypertension [60,61]. By diminishing central sympathetic discharges, these agents reduce the total peripheral resistance to lower the systolic and diastolic blood pressure [62]. Heart rate and cardiac output are transiently decreased with rilmenidine but not with moxonidine. However, the baroreflex sensitivity and renal function are preserved to minimize orthostatic hypotension [63,64]. Indeed, clonidine-type agents potentiate baroreflex vagal bradycardia, but these actions are related to their α_2-adrenoceptor activity [65,66]. With clonidine, this effect persists with chronic treatment [67]. The diminished baroreflex control of heart rate in hypertensive subjects is of concern since studies have shown that this is an independent risk factor for sudden death after acute myocardial infarction [68,69]. Hypertensive subjects have reduced heart rate variability and increased blood pressure variability [70], which is a significant risk factor for cardiovascular events [71]. One of the major acute effects of these agents is a reduction in blood pressure variability [72,73], although this has not always been observed with chronic treatment [74].

While rilmenidine has a plasma half-life of eight hours, the associated falls in plasma catecholamines, renin and antidiuretic hormone lead to a 24-hour control of arterial pressure [75,76]. Moxonidine induces a fall in arterial pressure that lasts 12 to 24 hours, despite its short plasma half-life of two hours [77-79]. Thus, there is no rebound hypertension after ceasing the therapy with either agent and the incidence of dry mouth and tiredness is common only during the first several weeks of the therapy [80-83].

One of the important issues in treating hypertension is the reduction of the secondary changes, such as cardiac and vascular hypertrophy. Clonidine as well as the second generation agents rilmenidine and moxonidine effectively regress left ventricular hypertrophy [84-86]. There is also an associated persistent reduction of atrial natriuretic peptide levels, which may suggest a favorable influence of the drug with the biosynthetic properties of ventricular myocardium [87]. Recently a reduction in ventricular natriuretic peptide transcription has been observed in obese SHR reflecting beneficial effect of moxonidine on the heart [88].

The overall clinical acceptability of rilmenidine and moxonidine is either similar to or better than the agents currently listed in the first-line treatment for hypertension [89-95]. However, the major limiting factor for continuing use of these agents has been a lack of large outcome studies with comparison to other agents [96].

I_1-Imidazoline Receptors in Renovascular Hypertension

As mentioned above, the importance of the sympathetic nervous system in human essential hypertension is well established. However, its role in secondary forms of hypertension such as renovascular disease is not so clear [97]. Renovascular hypertension is primarily due to renal ischaemia which alters the kidney's ability to excrete salt and fluid as well increasing the release of renin from juxtaglomerular cells in the kidney [98]. The resulting hypertension is an attempt to restore renal function. While the incidence of renal hypertension in the community is relatively low, its importance is increasing as our population ages, and the incidence of renal artery atherosclerotic lesions becomes more prevalent [99]. Perhaps surprisingly, many studies have established that the sympathetic nervous system plays an increasingly important role in maintaining the hypertension [100-103]. Thus there is very good argument that centrally acting sympatholytic agents like clonidine, may be useful in the treatment of renovascular hypertension [104]. However, there have been very few studies exploring this possibility. Sattar and colleagues have shown that a structural clonidine analogue, AL-12, normalizes blood pressure in 2K1C hypertensive rats [105]. Clonidine itself reverses the development of low-dose angiotensin II–induced hypertension [106]. Armah and colleagues have reported that moxonidine and clonidine lower blood pressure in renal hypertensive rats and dogs [107]. However, there have been no studies examining the second-generation agent rilmenidine in renovascular hypertension. Furthermore, there have been no comparisons with control animals to determine whether the responses are greater or less than one would expect in the absence of high blood pressure.

We examined whether there is evidence for an increased role of the sympathetic nervous system by giving an intravenous dose of rilmenidine to normotensive, 3 week and 6 week two-kidney-one-clip (2K1C) hypertensive rabbits [108]. The fall in blood pressure was substantially greater in the hypertensive groups compared to the normotensive rabbits. The bradycardia was similar in all three groups of rabbits. However, the sympatho-inhibition in the hypertensive animals was markedly less than in the normotensive rabbits. The acute depressor effect of rilmenidine was likely to be mediated by the reduction in RSNA, but there was no change in plasma renin activity. These studies suggest that the contribution of the sympathetic nervous system has increased in renovascular hypertension, which is consistent with

previous studies from other laboratories. However, what is surprising is the lack of enhanced sympathetic activity to the kidney and a reduced inhibition of RSNA by rilmenidine. It is possible that the sympathetic drive to other beds is enhanced in renovascular hypertension, but the evidence is not convincing except for the cardiac sympathetic activity, which is unlikely to account for the enhanced rilmenidine effect on blood pressure. Our studies have demonstrated that centrally acting agents like rilmenidine are appropriate for the treatment of renovascular hypertension and are significantly more effective than in normal animals [108].

Metabolic Effects of I_1-Imidazoline Agents

Hypertension is often observed as part of a more complex combination of diseases, such as obesity, hyperlipidaemic and hyperinsulinaemia, which are collectively known as syndrome X [109]. Obesity is associated with increased sympathetic nervous system activity [110] particularly to the kidney and therefore would be a most appropriate condition to treat with imidazoline selective agents [111,112]. To date, most clinical trials have involved moxonidine, which has been found to improve insulin sensitivity, reduce glucose intolerance and ameliorate high cholesterol levels [113,114]. In experimental studies, the obese SHR has been developed, which displays many of the characteristics of the human syndrome X [115]. Recent studies showed that moxonidine and rilmenidine acutely normalized blood pressure but worsened glucose tolerance through an α_2-adrenoceptor mediated effect. However, chronically both drugs improved glucose tolerance, lowered fasting insulin and reduced triglycerides and cholesterol [116]. The α_2-adrenoceptors in the pancreas reduced insulin secretion and increase glucagon release, while activation of I_1 receptors had the opposite effect. The beneficial metabolic effects of second generation imidazoline agents suggests that they may provide a very useful therapy, which may be further enhanced by the development of more selective I_1 agents.

Renal Effects of I_1-Imidazoline Agents

It is well described that clonidine like agents produce natriuresis and diuresis [117] and that their direct renal action is a distinct possibility. For example, natriuresis following imidazoline agents has been shown to be independent of renal nerves [118] and involve atrial natriuretic peptides. However, the separation of the central sympatho-inhibitory actions has been surprisingly difficult. Extensive studies in anesthetized rats from the Smyth laboratory have found evidence for both central and renal sites of action [119]. However, other groups have failed to find any actions of imidazoline agents that could not be ascribed to renal α_2-adrenoceptors [117,120,121]. By using a combination of central, systemic and renal administration to rats with one kidney and separate ureter collections, Smyth and colleagues found that moxonidine did not increase urine flow rate from the side where the nerves were denervated suggesting no direct renal action [122]. Together these studies suggest little role for imidazoline receptors in directly controlling renal function. However, imidazoline agents may indirectly affect water and sodium balance as rilmenidine has been shown to reduce salt appetite in SHR [123]. Further, administration of moxonidine into the medial septal area has been found to act on imidazoline receptors to inhibit water intake but acts on α_2-adrenoceptors when injected into the lateral ventricle to inhibit sodium and water intake [124]. Thus, central imidazoline actions may regulate water and sodium balance not only centrally through renal nerve activity but also by influencing forebrain structures to alter fluid intake.

I_1-Imidazoline Receptors in Heart Failure

It is well established that sympathetic activation in heart failure is intrinsically linked to disease progression and to adverse outcome [125]. Therefore, one promising strategy in the management of heart failure could be inhibition of sympathetic overactivity by stimulating I_1 receptors and α_2-adrenoceptors located within the RVLM [126-128]. Clonidine has recently been used for this purpose by Grassi and colleagues [129]. These investigators found a 26% reduction in muscle sympathetic burst frequency and a 47% fall in plasma norepinephrine concentrations after a 2-month administration of transdermal clonidine, without adverse alterations in cardiac function. Given that rilmenidine and moxonidine have fewer side effects than clonidine, the hope has been that this strategy might confer greater long-term clinical benefit and patient acceptance than widely used ß-adrenoceptor blockade [130]. There are very few studies in experimental animal models of heart failure to validate this view apart from one study using a 3 week treatment with moxonidine following coronary occlusion [127]. Early studies using a single dose of moxonidine in heart failure patients looked promising as the drug reduced afterload and was well tolerated [131]. A limited 11 week trial was also successful using a sustained release formulation of moxonidine at 0.9 mg bid to markedly reduce plasma norepinephrine concentration [132]. Consequently, the Moxonidine Congestive Heart Failure (MOXCON) trial, a randomized double-blind, placebo-controlled trial, was initiated in 425 centers in 17 countries with a plan to enter 4533 patients with class II-IV heart failure and a reduced ejection fraction [133]. Moxonidine sustained-release or matching placebo was titrated to a target dose of up to 1.5 mg BID (titrated from 0.25, 0.5,1 and 1.5 mg). The highest dose was nearly twice the dose used in the early trail by Dickstein and colleagues. Unfortunately, an early increase in death rate and adverse events in the moxonidine group led to premature termination of the trial because of safety concerns after 1934 patients were entered. The suggested reason was a too rapid and too severe inhibition of the sympathetic nervous system and the monitoring group recommended a slower dose titration and to not use the highest dose [133]. In support of this, the major time for excess deaths in the moxonidine group was during the dose titration phase. However, in the end the sponsor decided to terminate the trial. The fundamental question raised by these observations is whether excess of early mortality and morbidity in the MOXCON studies were dose related and due to excessive sympathetic inhibition, to intense rebound surges in sympathetic drive, heart rate, and blood pressure in occasionally noncompliant patients, or to both of these dose-related mechanisms [130,133,134]. Until the reasons of excessive mortality in the MOXCON trial are determined, further investment in this

potentially promising class of drugs for heart failure remains in question.

New Selective I_1-Imidazoline Agents

Until 1996, the most selective imidazoline ligand used was moxonidine that has only a 33-fold selectivity over α_2-adrenoceptors (Table **1**). This was changed with the development of a novel isofuran derivative AGN 192403 synthesized by Allergan and found to be an imidazoline ligand with a 500 fold selectivity over α_2-adrenoceptors [135]. AGN 192403 is equipotent to moxonidine in imidazoline receptor binding assays, 5-fold less potent that clonidine at the I_1 site, with less than 20 μM affinity for α_2-adrenoceptors [135] (Table **1**). However, AGN 192403 has been found to be an antagonist in a number of studies [136,137] [138,139] and had no effect on blood pressure when injected intravenously in monkeys and rabbits [135]. Accordingly, our own preliminary studies did not show a clear hypotensive action of this agent in conscious rabbits (unpublished observations, Head and Lee).

Benazoline is an imidazoline compound derived from cirazoline and one of the first of a series of compounds generated by the Universita di Camerino, Italy in collaboration with the Bousquet laboratory in Strasbourg [140]. It shows a very high (2000 fold) selectivity for imidazoline receptors over α_2-adrenoceptors in binding studies (Table **1**). However, when injected intracisternally to anesthetized rabbits produces a marked and somewhat unexpected hypertensive effect [141]. The reasons for such varied effects of imidazoline receptor agents remains to be elucidated but the hypertension was prevented by idazoxan and did not involve α_2-adrenoceptors [141].

BU98008 (1-(4,5-dihydro-1H-imidazol-2yl)isoquinoline hydrochloride) is a novel isoquinoline derivative which shows a 300 fold selectivity for I_1 receptors compared to α_2-adrenoceptors in rat brain membranes. While it has such high selectivity, its potency is about 10 times lower than rilmenidine. The agent also has very low affinity for I_2 receptors, with a 30 fold selectivity for I_1 over I_2 [142]. The agent was developed at the University of Bristol and was tested for hypotensive activity with a range of oral doses in conscious SHR. Only 1 mg/kg produced a small hypotensive effect 2-3 hours after administration but higher or lower doses were ineffective [142]. It is not known whether this agent is an agonist or antagonist or whether the lack of convincing hypotensive effects was due to limited bioavailability or dosing regimes. Clearly a much more comprehensive evaluation of this agent is warranted.

A range of recent new selective agents has come from the laboratory of Bousquet and colleagues in Strasbourg. Initial reports suggested that an imidazoline derivative called LNP509 is a selective imidazoline agent with virtually no affinity for α_2-adrenoceptors. However, it is not overly potent (IC50 of 0.5 mmol) and calculations of its selectivity are therefore difficult. The ratio of IC50 suggests that it is somewhat greater than 20. Administration of LNP509 intracisternally in anesthetized rabbits produced a decrease in blood pressure in a dose-dependent manner [47]. This agent reduces blood pressure when injected into the RVLM, but unfortunately does not cross the blood brain barrier [47]. Thus, we cannot conclude from these studies whether a selective action of LNP509 on medullary imidazoline receptors alone is sufficient to produce hypotension. Injection of a very low dose of LPN509 markedly facilitated the hypotensive effect of a highly selective α_2-adrenoceptor agonist suggesting that there was a marked facilitation between the imidazoline receptors and α_2-adrenoceptors. This effect was not observed in D79N mice that lack a functional α_2-adrenoceptor. Recently LPN509 was shown to dilate the microcirculation of SHR and lower blood pressure through a reduction of cardiac output and total peripheral resistance, an effect paralleled by rilmenidine but not clonidine [143].

A new agent LPN 640 is similar in its cardiovascular actions to LPN509 but is more lipophylic and does cross the blood brain barrier [15]. S23757 is an agent developed for SERVIER laboratories and has a much higher (100 fold) affinity for I_1 receptors than LPN509 also without appreciable α_2-adrenoceptors activity. It is also lipophilic and its selectivity is greater than 1900 for I_1 over α_2-adrenoceptors (Table **1**). While the hypotensive actions of these highly selective agents have been documented [15], no full description of their cardiovascular actions has appeared to date in the literature. Another agent announced is an azido derivative of LPN911, which is a new iodine labeled imidazoline probe with a 50000 selectivity for I_1 receptors and which binds to these receptors in a non-reversible manner [15]. Finally, a novel I_1 antagonist S23515 has been synthesized, which is similar to LPN640 in its high selectivity for I_1imidazoline receptors [15]. Thus there does now appear to be a range of agents that are selective agonists and antagonists at I_1 receptors. However, while we await the publication of the cardiovascular effects of these latest agents, there has not been to date a clear case made that these third generation I_1 agents can lower blood pressure through a specific I_1 receptor mechanism. This is based on the results from AGN 192403, benazoline, BU98008, which either have no effect on blood pressure or are hypertensive agents.

I_2 IMIDAZOLINE RECEPTORS

Imidazoline Binding Proteins

The I_2 binding sites have originally been characterized using [^{3}H]-idazoxan and termed as IGRS [144]. The I_2 sites have first been located on the outer membranes of mitochondria and identified as allosteric sites on MAO A (~61-kD) and MAO B (~55kDa) [145,146]. More recent evidence suggests, however, that not all I_2 binding sites are present on MAO [14]. One MAO-unrelated I_2 binding protein has recently been isolated from the rabbit brain and identified as a 45-kD protein brain creatine kinase [147]. Another I_2 binding protein (~28-kDa) was still observed in the liver of MAO-knockout mice [148]. Although the latter protein was not identified chemically, it does not appear to be creatine kinase due to the lack of significant levels of this enzyme in normal liver [149]. The other enzymes that also show affinity for I_2 binding sites have been reported to comprise several copper-containing amino oxidases, including soluble semicarbazide-sensitive amine oxidase (SSAO) [19]. Further, the nature of the association between I_2 sites and

MAO has not been clarified, as the I_2 binding sites appear to exist within only a subset of MAO molecules [150]. This may partly explain why, in contrast to earlier studies reported in the brain and liver [145,151], I_2 density and MAO-B activity are only weakly correlated in platelets [152]. Moreover, although some I_2 selective ligands have been shown to inhibit MAO activity [153], a recent studies using a novel irreversible ligand, BU99006, found little modulation of MAO activity and MAO binding density due to its binding to I_2 sites [154].

I_2 Imidazoline Receptors and Depression

Evidence indicates that the I_2 binding sites can be used to modulate behavioural states such as depression and anxiety [155]. Interestingly, alterations in I_2 receptor density have been reported in depressed patients [151]. Thus, imidazoline compounds may have therapeutic value for treating depressive illness [156]. Animal studies seem to be in accord with this possibility, indicating the role of I_2 receptors in modulating levels of central catecholamines [157,158]. A comprehensive review by Halaris and Piletz have summarized the last decade of progress for imidazoline receptors and agmatine in Psychiatry. They suggest that while I_2 ligand are of interest as antidepressants, they are not potent enough to be useful and that more potent agents will need to be developed [159].More recently, it has been reported that a selective I_2 ligand BU224 with nanomolar affinity for I_2 receptors, increased 5-HT levels in the frontal cortex and hypothalamus of rats exposed to forced swim test, and reduced their immobility in this test, suggesting antidepressant-like activity [160]. Further, administration of BU224 and idazoxan, but not selective α_2-adrenoceptor antagonist RX821002 has been shown to potentiate the corticosterone response to restraint stress in rats, indicating that I_2 binding sites may also play a role in modulating the HPA axis response to acute psychological stress [161].

I_2 imidazoline Receptors and Feeding

Another important functional role of I_2 binding sites is the modulation of feeding behavior, since idazoxan and other ligands for I_2 sites have been shown to elicit hyperphagia in rats [162,163]. This effect appears to be in contrast with moxonidine-induced hypophagia in obese SHR [111] and Zucker rats [164], thus indicating another functional difference between the two I-receptor subtypes. The molecular mechanisms of the hyperphagic effect of I_2 ligands have not been associated with changes in MAO activity [165] and thus remain elusive. Moreover, a recent finding that idazoxan elicits a more potent hyperphagic effect in rats than metrazoline and benazoline, although its affinity for I_2 binding sites is lower than that of metrazoline and similar to that of benazoline, raises the question of whether its orexinergic effect might also be due to interaction with other receptors [163]. It is noteworthy that, in the same study [163], injections of idazoxan or metrazoline into the third, fourth or lateral brain ventricle did not evoke hyperphagia, suggesting a peripheral rather than a central site of their orexinergic action. However, further research is necessary to determine more precisely the sites and mechanisms of hyperphagic action of I_2 compounds.

New Ligands for I_2 Imidazoline Receptors

One difficulty in studying the function of I_2 binding sites is that the high-affinity ligands for these sites still have some affinity for α_2-adrenoceptors. Recently, a new series of four 2-(benzofuranyl)-2-imidazoline (2BFI) derivatives were investigated as potential ligands for imaging brain I_2 receptors using positron emission tomography [166]. At least two, BU20012 and BU20013, retained high affinity and moderate selectivity and penetrated the brain when administered peripherally in the mouse. In addition, isothiocyanate form of 2BFI (BU99006) has been synthesized which binds irreversibly to I_2 sites and thus is extremely useful both *in vivo* and *in vitro* studies [167]. With the use of this selective I_2-alkylating agent, it has been shown that I_2 sites had a relatively short half-life of ~2 hours in mice and 4 hours in rats [154]. Also, it has been found that 30-kD and 45-kD proteins, but not the 43-kD and 85-kD proteins, immunodetected using an antibody raised against an ~70-kD idazoxan/clonidine binding protein (which recognized both I_1 and I_2 binding sites) are related to I_2 binding sites in the mouse brain [168].

I_3 IMIDAZOLINE RECEPTORS

There is now a considerable body of evidence to suggest that imidazoline compounds can induce insulin secretion from pancreatic β cells, which is not mediated by I_1 or I_2 binding sites [see reviews by 14,20]. The binding site that mediates this action of imidazolines has been classified as I_3 site, although the structural requirements for binding of ligands to this site have not been fully defined. The I_3-mediated insulotropic action of imidazolines has first been ascribed exclusively to the closure of ATP-sensitive potassium channel (K_{ATP}) leading to depolarization, calcium influx and release of insulin [169].

More recently, it has been shown that the imidazoline agents can be divided into 2 groups. The first (classic) group of agents, such as RX871024, has insulotropic activity at both normal and elevated glucose levels, which is mediated *via* the closure of K_{ATP} channel. By contrast, a new generation of agents has only the glucose dependent insulotropic activity without affecting K_{ATP} channels and can directly affect insulin exocytosis [20,170]. For example, imidazoline compound BL11282 (LY374284) has recently been shown to restore biphasic insulin secretion in pancreatic islets of diabetic db/db mice by amplifying glucose-induced insulin secretion at a site distal to Ca^{2+}-influx [171]. Similarly, stimulatory action of (+)-2-(2-(4,5-dihydro-1H-imidazol-2-yl)-thiopene-2-yl-ethyl)-pyridine (NNC77-0074) on insulin secretion was not associated with membrane depolarisation or a change in the activity of K_{ATP} channels, while NNC77-0074 potently inhibited glucagon secretion from rat islets [172]. These actions were exclusively exerted by modulation of exocytosis of the insulin- and glucagon-containing granules. Another new imidazoline compound 2-[173]-pyridine (NNC77-0020) has been found to modulate pancreatic hormone secretion in a similar fashion, comprising glucose-dependent stimulation of insulin and somatostatin secretion and inhibition of glucagon release [173]. This dependence on ambient glucose concentration may provide

an ideal basis for the development of novel imidazoline-containing anti-diabetic compounds. In particular, these compounds may significantly reduce the risk of hypoglycemic episodes, associated with sulfonylureas, which are widely used in the management of type 2 diabetes mellitus.

The specificity of insulinotropic effects of imidazolines in insulin-secreting cells remains to be firmly established. A recent study demonstrated that [^{3}H]clonidine binding showed a high and low affinity sites in insulin secreting HIT cells [174]. The low-affinity site has been shown to be the pore-forming subunit of the K_{ATP} channel (Kir6.2 deltaC26). The ion channel-blocking alkaloid, quinine displaced [^{3}H]clonidine with both low-affinity (83% of sites) and high-affinity sites (17% of sites) in insulin-secreting HIT cells, indicating that there are imidazoline binding sites in these cells which also recognize quinine. The existence of distinct I_3 binding proteins has also been suggested on the basis of pharmacological experiments, which demonstrated that although I_3 agonist, efaroxan, and the β-carboline, harmane, directly elevate cytosolic Ca^{2+} and increase insulin secretion, these responses display different characteristics [175]. Nonetheless, it has recently been shown that harmane activates at least two distinct mechanisms to promote insulin release. One of these may involve binding to imidazoline I_3-receptors, while a second arises from the interaction of harmane with ryanodine receptor-1, leading to the generation of sustained Ca_{2+} oscillations [175,176] [177]. Either way, it appears that β-carbolines can potentiate the rate of insulin secretion from human islets and thus may be useful prototypes for the development of novel insulin secretagogues [176].

RECEPTOR PROTEINS FOR IMIDAZOLINES

Several protein candidates for the I_1-imidazoline receptor have been reported. The first candidate, known as imidazoline receptor binding protein (IRBP), was a 70 kDa protein isolated from bovine adrenomedullary cells which has nanomolar affinity for imidazolines but not for catecholamines [178]. Polyclonal antibodies generated against the IRBP can detect both neuronal (I_1) and glial (I_2) imidazoline sites in the rat brain [179]. IRBP antiserum also detects a 33 kDa protein in human platelets which was found to be correlated with the density of I_1-imidazoline sites [180]. Other studies from the Bousquet laboratory have detected a 43 kDa imidazoline binding protein in the human brainstem using anti-idiotypic antibodies, which was shown to have the properties of both I_1- and I_2-imidazoline receptor subtypes [181]. Known as anti-idiotypic imidazoline receptor protein (AIRP) antiserum, this antiserum potently inhibits the I_1-imidazoline binding of ^{3}H-clonidine [182,183].

Both the IRBP and AIRP antisera detect an 85-kDa protein band on Western blots [184]. Tissue levels of the 85 kDa protein on Western blots were shown to be nearly identical using either IRBP or AIRP antiserum. Furthermore, it is now appreciated that all these bands may be products of a precursor protein named IRAS [16,185], also known as Nischarin [186]. IRAS was isolated from a human hippocampal cDNA library some years ago with the clone selected using two different antisera developed against imidazoline receptors. IRAS cDNA encodes moxonidine binding sites in transfected cells along with other properties of an I_1-imidazoline receptor [16,17,187]. Based on its unique internal amino acid sequence, an epitope-selective antiserum against human IRAS was produced (P1209) which selectively detects the full-length form of the protein on Western blots. It has further been shown that an 85 kDa protein can be co-immunoprecipitated from cellular extracts along with the full-length form of IRAS [185]. Therefore, all three of these antisera (IRBP, AIRP and P1209) appear to detect various forms of IRAS or closely related proteins; but which protein (33 kDa to 85 kDa, or larger) acts as an imidazoline receptor has remained an open question [185].

Recent developments with the cloning of the imidazoline receptor were mainly related IRAS which have now shown that transfection into cell lines leads to the appearance of non-adrenergic clonidine binding sites [17]. Furthermore, Piletz and colleagues have found in a growth-arrested PC-12 cell line stably transfected with IRAS, expresses a lower basal and nerve growth factor-stimulated level of the activated form of extracellular receptor kinase (ERK) than found in a vector-only transfected control. These findings suggest that IRAS is a membrane-associated mediator of receptor signaling [17]. A consistent observation was that IRAS has important biological effects on three interrelated cell functions: insulin and growth-factor mediated cell growth, integrin mediated cell-shape, and protective responses to various apoptotic stimuli [188]. The findings with IRAS suggest that the I_1 receptor may actually be a constitutively active enzyme with an allosteric binding site for moxonidine and allied agents. It was further suggested that the actions of these imidazoline compounds might be partially mediated through neurite outgrowth changes. The human IRAS protein has been shown to contain a phox domain giving it the functionality of a sorting nexin. As such, IRAS may be involved in protein sorting, signal transduction and vesicle fusion [189].

One of the other key new findings in this area has been the isolation of a 45 kDa protein rabbit brain by affinity chromatography using a highly selective ligand for I_2-imidazoline receptors [147,166]. While I_2-imidazoline binding affinity has generally been ascribed to an allosteric binding site on monoamine oxidase [146], the 42 kDa protein has been found to be creatine kinase. These findings suggest a possible novel link between metabolic function of neurons, imidazoline ligands and cardiovascular activity.

ENDOGENOUS LIGANDS: AGMATINE

One of the interesting aspects of imidazoline research has been the search for endogenous ligands. In 1984 Atlas and colleagues partially purified a substance from mammalian brain, which displaced clonidine from α_2-adrenoceptors and which they called CDS [190]. This substance also displaced I_1 and I_2 imidazoline binding [191,192]. In 1994 Li and colleagues described agmatine, which is a polyamine, found in mammals and which is formed by decarboxylation of L-arginine by arginine decarboxylase (ADC) in mammalian tissues and binds to α_2-adrenoceptors and imidazoline receptors [22]. While agmatine is not the classic CDS [193],

previous studies have suggested that agmatine is a neurotransmitter within the central nervous system [194]. Agmatine is contained in vesicles in neuronal cytoplasm in many regions of the brain including the hippocampus and hypothalamic nuclei and is also released from neurons upon depolarization [194]. The CNS also contains enzymes for production and breakdown of agmatine. In addition, agmatine can also block N-methyl-D-aspartate (NMDA) receptors and other ligand-gated cation channels as well as inhibit nitric oxide (NO) synthase [for review see 195]. Thus, agmatine, an endogenous cationic amine, exerts a wide range of biologic effects, but its physiologic role is still to be determined. The field of agmatine research has certainly expanded over the last few years.

Agmatine Metabolism

Although the cloning and sequencing of ADC from plant and bacteria have been reported extensively, until recently the structure of mammalian enzyme has not been known. Using homology screening approach, Zhu and colleagues identified a human cDNA clone that exhibits ADC activity when expressed in COS-7 cells [196]. It is well established that agmatine in vertebrates may be derived from multiple sources, including the diet and endogenous synthesis *via* ADC. However, agmatinase is thought to be the only enzyme specific for agmatine catabolism [197]. In humans, agmatinase mRNA is most abundant in the liver and kidney but also is expressed in several other tissues, including brain [198]. Its expression in human liver is induced during hepatitis B virus infection, suggesting that agmatinase may play a role in the pathophysiology of this disease. There is little information regarding how much agmatine in mammalians is catabolized by agmatinase versus other enzymes such as diamine and amine oxidases. Surprisingly, comparisons of primary sequences of several vertebrate agmatinases demonstrate that the agmatinase in mice has little or no catalytic activity [199]. This not only raises questions about the physiologic routes of agmatine disposal in mice, but also suggests the existence of specific differences in mechanisms for regulating agmatine levels.

Neuroprotective Effects of Agmatine

Among several biologic effects, the ability of agmatine to protect against ischemic injury, neuropathic pain and seizures is particularly intriguing [200-202]. It is thought that the synthesis of agmatine and NO production could be reciprocally regulated by inflammatory stimuli and agmatine might be an endogenous anti-inflammatory agent [203,204]. Recent studies show that agmatine inhibits NO production by decreasing the activity of inducible NO synthase (iNOS) in macrophages and astroglial cells by decreasing the levels of iNOS protein, which may provide a molecular basis for the neuroprotective and anti-inflammatory actions of agmatine [205]. The neuroprotective actions of agmatine have recently been examined in cortical neuronal cell cultures and also in a model of the middle cerebral artery occlusion in mice. The results suggest that agmatine may also act as a competitive inhibitor of neuronal NO synthase (nNOS) and can protect neurons from ischemic injury in both *in vitro* and *in vivo* models [206].

Agmatine is also protective against cell death induced by NMDA excitotoxicity *in vitro* [207]. Systemic administration of agmatine significantly reduced the severity of a spontaneous pain-like behavior elicited by intraspinal injection of the AMPA/metabotropic receptor agonist quisqualic acid in rats [208]. However, agmatine is not protective against cell death induced by protein kinase blockade or increase in cellular calcium [207].

It has been shown that agmatine reverses persistent pain induced by inflammation, neuropathy, and spinal cord injury, without producing antinociceptive effects in acute pain tests in rodents [201,209]. Recent studies in nNOS knockout mice suggest that agmatine inhibits morphine physical dependence and potentiates morphine analgesia in part *via* a nNOS-dependent mechanism [210]. It is likely that the mechanism of its antinociceptive action is also site specific, because of the agmatine-induced potentiation of morphine analgesia has been shown to depend on imidazoline receptors at spinal and both imidazoline- and α_2-adrenoceptors at superspinal sites in mice [211]. Overall, considering that agmatine has been shown to cross the blood-brain barrier readily [212], these studies indicate that agmatine may be a novel therapeutic strategy to reduce ischemic injury, pain and seizures.

Agmatine and Cell Proliferation

Recent evidence suggests that agmatine in the chyme of the gut is likely to represent an essential source of agmatine in the tissues of the organism [213]. Exogenous agmatine is unevenly distributed among the organs, as estimated by accumulation of radioactivity in organs in tissues after [214]-agmatine ingestion, being mostly accumulated by the liver (67% of radioactivity) [215]. In view of these findings, the effect of agmatine on proliferation of tumor and non-tumor cells has been determined in the liver and intestinal tissues [213]. It has been found that an increase in the availability of gastrointestinal agmatine for absorption impairs liver regeneration and may contribute to the development of liver diseases. Agmatine inhibited the proliferation of six human intestinal tumor cell lines in a concentration-dependent manner; this inhibition probably was attributable to an interaction between agmatine and the intracellular polyamine system [216]. Specific accumulation of [214]-agmatine tested in six human intestinal tumor cell lines and in the glioma cell line SK-MG-1 was mediated by a specific agmatine transporter, rather than by amino acid or monoamine carriers, by the putrescine carrier, by 5-HT_3 receptor channels, by Ca^{2+} channels or by the organic cation transporters [217]. Together, these findings suggest that agmatine can be absorbed from the gut by means of a specific transport system, which regulates the intra- and extracellular concentration of agmatine in humans.

Antidepressant and Anxiolytic Effects of Agmatine

There is limited evidence that agmatine might also be important as antidepressant and anxiolytic agent. Agmatine concentrations have been shown to be increased in the plasma of depressed patients, while treatment with the antidepressant bupropion normalized plasma agmatine levels [218]. Endogenous agmatine in plasma was increased in

response to cold-restraint stress in rats, which indicates that agmatine may also play a role in modulating acute stress reactions [219]. Further, agmatine decreased immobility time in the forced swim test and increased the time spent in the open arms in the elevated plus maze in rats, further indicating that endogenous agmatine may have modulatory effect on anxiety and depression [220]. However, the mechanism of antidepressant-like effects of agmatine, when assessed in the forced swimming test in mice, involved an interaction with NMDA receptors, NO and α_2 -adrenoceptors [221]. Thus, given that agmatine can also exert such diverse effects, the involvement of I-receptors in its antidepressant effects remains to be firmly established.

ENDOGENOUS LIGANDS: IMIDAZOLEACETIC ACID-RIBOTIDE (IAA-RP) AND HARMANE

George Prell and colleagues have recently described the isolation and properties of IAA-RP as an endogenous CDS [8]. This agent is present in the brain in neurons and is found in extracts containing CDS. IAA-RP displaces clonidine from bovine brain preparations with nM affinity and can induce arachidonic acid release from PC12 cells like moxonidine. IAA-RP is rich in the brainstem regions that are known to be sites of clonidine action, such as the RVLM. This group has proposed that IAA-RP is the endogenous clonidine. However, when IAA-RP was injected into the RVLM of rats, blood pressure increased. While this is similar to the actions of some CDS preparations [222], it is unclear how this occurs since microinjection of clonidine into the RVLM is well known to decrease blood pressure.

Another group of putative endogenous imidazoline ligands are represented by harmalan and harman, which are β-carbolines [223]. They displace clonidine with modest affinity and have high affinity for imidazoline sites. Unlike CDS or IAA-RP, harmane injected into the RVLM of anesthetized rats lowers blood pressure, an effect which is blocked by efaroxan [224]. Several β-carbolines have been found to potently stimulate insulin release from human islet cells, an effect blocked by the I_3 antagonist KU14R [175]. However, unlike efaroxan, harmane failed to release insulin from freshly isolated cells. This suggests that the harmane-induced release of insulin is not solely mediated by I_3 sites in the beta cells.

IMIDAZOLINE RESEARCH: THE FUTURE

Perhaps the over-arching message from the field of imidazoline research is that there is not one future but many. With each new discovery, a new turn is taken and rarely does one area ever close. With the various receptors now being discovered as enzyme and protein targets, with endogenous receptor proteins being involved in cell signaling and with at least 3 classes of endogenous ligands there is indeed much to find out. The challenge will be to take these new findings into the clinical settings with novel therapeutic agents. There are some older challenges that remain, including the definitive description of the I_1 receptor and the realization of truly selective third generation imidazoline agents that are indeed able to lower blood pressure in their own right.

ABBREVIATIONS

GABA	=	γ-Aminobutyric acid
α-MNA	=	α-Methylnoradrenaline
2MI	=	2-Methoxyidazoxan
AIRP	=	Anti-idiotypic imidazoline receptor protein arginine decarboxylase
K_{ATP}	=	ATP-sensitive potassium channel
CDS	=	Clonidine displacing substance
ERK	=	Extracellular receptor kinase
IAA-RP	=	Imidazoleacetic acid-Ribotide
IRAS	=	Imidazoline receptor antisera selected
IRBP	=	Imidazoline receptor binding protein
IGRS	=	Imidazoline-guanidinium receptive site
iNOS	=	Inducible NO synthase
MAO	=	Monoamine oxidase
MOXCON	=	Moxonidine Congestive Heart Failure
nNOS	=	Neuronal NO synthase
NMDA	=	N-methyl-D-aspartate
NA	=	Noradrenergic
PGSN	=	Pre-ganglionic sympathetic neurons
RVLM	=	Rostral ventrolateral medulla
SSAO	=	Soluble semicarbazide-sensitive amine oxidase
SAC	=	Spontaneously active cell
SHR	=	Spontaneously hypertensive rats
2K1C	=	Two-kidney-one-clip

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