

## **Imidazoline I<sub>1</sub> Receptors in the Ventrolateral Medulla and Their Role in Cardiorespiratory Control**

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### **I. The RVLM as the Site of Action for Central Antihypertensive Agents**

The probable site of the hypotensive action of clonidine is within the rostral ventrolateral medulla (RVLM). Localization of clonidine's action to the medulla oblongata arises from experiments showing that transection of the brainstem at the pons preserves the response to clonidine and its analogues, whereas transection of the spinal cord above the first thoracic segment abolishes it (1,2). Human patients with cervical spinal cord transection also fail to show a vaso-depressor response to clonidine and manifest only the direct pressor action on blood vessels (3). Furthermore, clonidine lowers blood pressure when given into the vertebral arteries at doses that are inactive when given into the carotid artery, even when the basilar artery is clipped to limit the injection to ponto-medullary structures (4). Interestingly, clonidine does not lower heart rate when given by this route, suggesting that the bradycardiac and vasopressor actions of clonidine are mediated by distinct neuronal sites.

Within the medulla of the rat, direct microinjection of clonidine or related analogues into the RVLM lowers blood pressure, but microinjections as little as 2 mm distant to the pressor area of the RVLM are without effect (5,6).

However, delivery of clonidine into the caudal ventrolateral medulla (CVLM) has been reported to lower blood pressure as effectively as RVLM microinjection, although with a longer delay to reach maximum effect (7). The site of action for clonidine has been mapped in the cat, a species with a larger medulla that lends itself more readily to localization studies (8). The largest effect of clonidine was observed in an intermediate area 2 mm caudal to the RVLM pressor area and at the same dorsoventral and lateral coordinates (8). Clonidine was assumed to stimulate neurons in this region, which in turn inhibit RVLM pressor neurons. A major French group has also noted that the active site for clonidine in the cat is caudal to the region from which sympathoexcitatory RVLM neurons have been recorded (9). Another group found two active zones for clonidine microinjections within the RVLM region, one located within the pressor zone and another area more caudal and medial (10). The two sites appear to be independent, since lesion of one allows an effect of clonidine at the other site (10). Thus, it appears likely that the action of clonidine is not confined to the region containing the cell bodies of tonically active sympathoexcitatory neurons and may involve other nearby groups of neurons.

Microinjection of clonidine into the nucleus tractus solitarius (NTS) will also lower blood pressure; 100-fold higher concentrations are required relative to the RVLM (11). Lesions of the NTS have no effect on the response to clonidine (12,13), but animals with RVLM lesions show only pressor responses to clonidine (14). Moreover, localized microinjection of the imidazoline  $\alpha_2$ -antagonist idazoxan into the RVLM prevents the vasodepressor effect of systemic clonidine (8,15,16) or rilmenidine (2). Thus, administration of an antagonist specifically into the RVLM, leaving intact the NTS and other potential sites of action, completely abolishes the hypotensive action of centrally acting imidazolines.

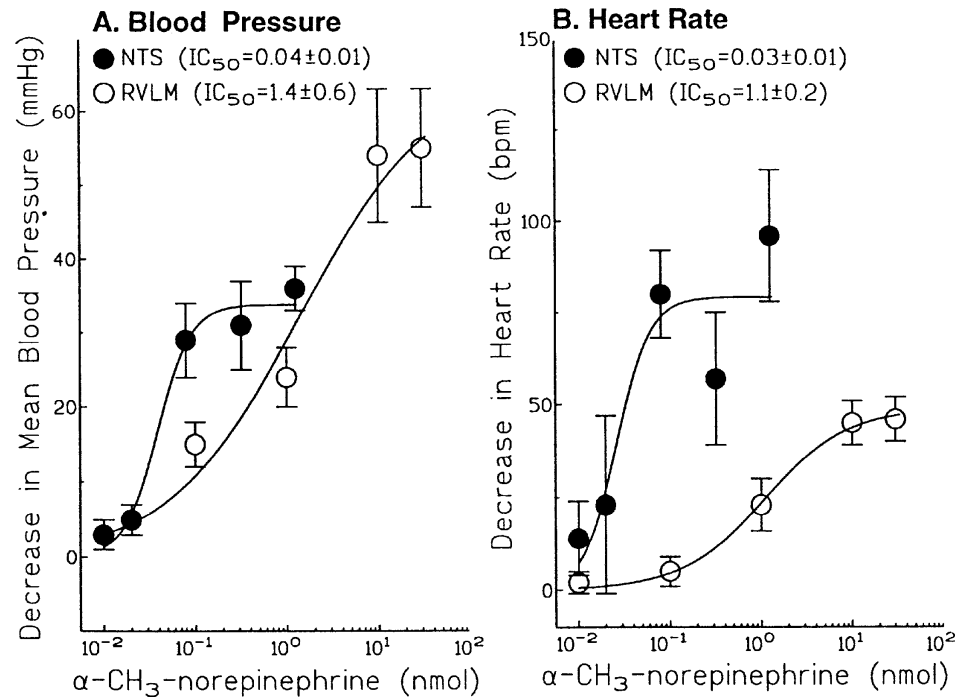
Clonidine elicits bradycardia by two mechanisms, one mediated by sympathoinhibition and the other mediated by an increase in vagal tone. There is some evidence that the vagal component of the heart rate responses might be mediated within the nucleus ambiguus. Microinjection of either norepinephrine or clonidine into the nucleus ambiguus elicits bradycardia with little effect on blood pressure (17). This bradycardiac response is abolished by vagotomy. In contrast, microinjection of clonidine into the nearby RVLM lowers blood pressure with little effect on heart rate, and this response is unaffected by vagotomy (17). Furthermore, microinjection of idazoxan into the nucleus ambiguus blocks the bradycardiac action of clonidine without affecting the blood pressure response, whereas delivery of idazoxan into the RVLM blocks the hypotensive action without affecting bradycardia (16). Most studies show that microinjection of idazoxan in the RVLM blocks the bradycardiac effect of imidazoline agents (8,15), but the spread of antagonist into the nucleus ambiguus cannot be ruled out.

## II. NTS $\alpha_2$ -Adrenergic Receptors as a Site of Action for Central Antihypertensive Agents

Activation of central  $\alpha_2$ -adrenergic receptors elicits falls in blood pressure and heart rate. The action of the nonimidazoline central antihypertensive agents—specifically  $\alpha$ -methyldopa, guanfacine, and guanabenz—is almost certainly attributable to an action of  $\alpha_2$ -adrenergic receptors. The most likely site of action for  $\alpha_2$ -agonist action in the brainstem is the NTS. Microinjection of minute doses (0.02 to 0.3 nmol) of norepinephrine, epinephrine, or  $\alpha$ -methylnorepinephrine, a selective  $\alpha_2$ -agonist which is the active metabolite of  $\alpha$ -methyldopa, into this brain region lowers blood pressure and heart rate (1,18–21). Blood pressure and heart rate are usually decreased by about the same proportion and there is no evidence for a dissociation of blood pressure and heart rate effects. The dose-dependent fall in blood pressure and heart rate elicited by  $\alpha$ -methylnorepinephrine microinjected into the NTS is shown in Figure 1 [solid symbols; data from Zandberg and colleagues (20)]. Vagotomy or treatment with atropine actually potentiates the hypotensive action of catecholamines within the NTS, indicating that  $\alpha_2$ -receptor activation inhibits sympathetic activity.

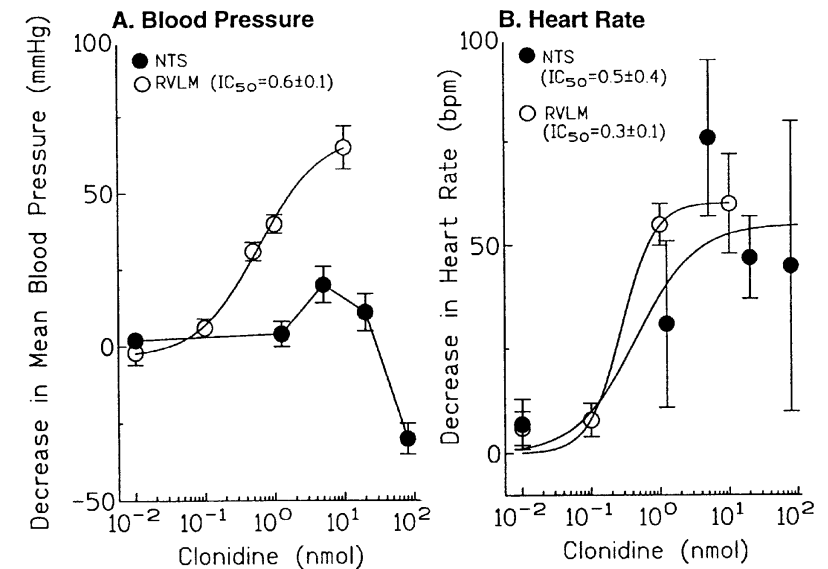
The imidazoline  $\alpha_2$ -agonist clonidine is much less effective than the catecholamines, having no effect at 1.25 nmol and decreasing blood pressure only 20 mm Hg at a 5-nmol dose, whereas epinephrine lowers blood pressure 24 mm Hg at a 600-fold lower dose (20). Higher doses of clonidine, up to 80 nmol, actually increase blood pressure, as shown in Figure 2. Note the contrast with the potent action of  $\alpha$ -methylnorepinephrine shown in Figure 1. Oxymetazoline is completely ineffective in lowering blood pressure when administered into the NTS in doses up to 20 nmol (20). In another study, clonidine was inactive at doses up to 30 nmol, whereas the effect of epinephrine was half-maximal at 3 nmol (22). The ineffectiveness of clonidine and oxymetazoline in lowering blood pressure from within the NTS is probably due to their status as partial agonists. Clonidine and oxymetazoline often act as  $\alpha_2$ -adrenergic antagonists, particularly in the presence of high concentrations of endogenous catecholamines (23), such as might be found in the NTS region. BHT 920, a noncatecholamine full  $\alpha_2$ -agonist, is about as potent as  $\alpha$ -methylnorepinephrine in lowering blood pressure when given into the NTS (21). Interestingly, clonidine is able to decrease heart rate when microinjected into the NTS (Fig. 2), implying that blood pressure and heart rate are independently regulated within the medulla.

A mapping study showed the active zone for  $\alpha$ -methylnorepinephrine to be within 0.5 mm of the caudal tip of the area postrema in proximity to the solitary tract (24). This corresponds to the area eliciting the greatest depressor response when stimulated. When delivered within the NTS,  $\alpha$ -methylnorepin-



**Figure 1** Dose-dependent cardiovascular actions of  $\alpha$ -methylnorepinephrine upon microinjection into the RVLM or the NTS. Data for NTS (solid circles) were reanalyzed from Zandberg and colleagues (20) and represent the decrease in mean blood pressure (panel A) or heart rate (panel B) at 5 min after bilateral microinjection in a volume of 500 nL. Data for the RVLM (open circles) were reanalyzed from Granata and colleagues (44) and represent peak decreases in blood pressure (panel A) or heart rate (panel B) after bilateral microinjection in a volume of 100 nL.  $IC_{50}$  values were determined by nonlinear curve fitting to a logistic equation by using the program InPlot (152) (Graphpad Software, San Diego, CA). The logistic function accounted for at least 92% of the total variance in each case.  $\alpha$ -Methylnorepinephrine is at least 30-fold more potent in the NTS than in the RVLM ( $P < 0.01$ ).

ephrine (0.5 nmol) not only decreases blood pressure and heart rate but also potentiates the depressor response to stimulation of the aortic depressor nerve (25). The  $\alpha_2$ -adrenergic antagonist yohimbine has the opposite effect, even at very low doses, attenuating the response to aortic nerve stimulation while abolishing the effect of  $\alpha$ -methylnorepinephrine (25). Microinjection of yohimbine into the NTS increases blood pressure in doses as low as 10 pmol, whereas the imidazoline  $\alpha_2$ -antagonist idazoxan is 25-fold less potent (19). These data demonstrate a potent  $\alpha_2$ -adrenergic receptor mechanism for regulating blood pressure within the NTS, which is integrated into the baroreflex and is tonically active, as indicated by the potent pressor response to antagonists.



**Figure 2** Dose-dependent cardiovascular actions of clonidine upon microinjection into the RVLM or the NTS. All details as in Figure 1. Clonidine had dose-dependent action on blood pressure only within the RVLM. The  $IC_{50}$  values for clonidine for barycardiac actions were identical in the NTS and the RVLM.

### III. Central Nervous System $\alpha_2$ -Adrenergic Receptors as Mediators of Central Nervous System Depression

While activation of  $\alpha_2$ -adrenergic receptors is an effective means of lowering blood pressure, it also causes sedation and other signs of central nervous system depression, which are the major side effects of centrally acting antihypertensive agents (26). Agents that act within the central nervous system to inhibit sympathetic nervous system activity have long been in use as antihypertensive agents (26). Clonidine and  $\alpha$ -methyldopa represent the first generation of centrally acting antihypertensive agents but have fallen out of use owing to a high incidence of side effects such as sedation and dry mouth. Extensive efforts to separate the vasodepressor actions from sedative effects were unsuccessful. Guanabenz and guanfacine were developed as more potent and selective  $\alpha_2$ -agonists; indeed, in binding studies, they show 3- to 10-fold higher affinity at  $\alpha_2$ -adrenergic receptors than clonidine, yet they are up to 10-fold less potent than clonidine as antihypertensive agents and elicit the same profile of side effects (26,27).

The sedative action of  $\alpha_2$ -agonists may be mediated within the locus ceruleus, a brain region critical in the control of alertness and sleep-wake cycles, because direct microinjection of clonidine or other  $\alpha_2$ -adrenergic agonists into

the locus ceruleus elicits sedation (28,29). Microinjection of pertussis toxin, an agent that functionally uncouples  $\alpha_2$ -adrenergic receptors, into the locus ceruleus attenuates the sedative action of clonidine and the altering action of the  $\alpha_2$ -antagonist yohimbine (28). The activity of neurons in the locus ceruleus neurons is potently inhibited by clonidine and by nonimidazoline  $\alpha_2$ -agonists, whereas rilmenidine, a central antihypertensive agent with reduced sedative action, inhibits activity in the locus ceruleus only at very high doses (30). Clonidine and other imidazolines have no effect on blood pressure when applied directly into the locus ceruleus, showing that this region is involved only in sedative side effects and not the vasodepressor action of these agents (20,31).

The evidence that  $\alpha_2$ -adrenergic receptors mediate sedation is strong, since the alkaloid antagonists yohimbine and rauwolscine potently and completely reverse the sedation elicited by clonidine (32,33), whereas higher doses are required to reverse the hypotensive action (9,32). Profound sedation is elicited by  $\alpha_2$ -agonists that are not imidazolines, including the antihypertensive agents  $\alpha$ -methyldopa, guanabenz, and guanfacine. In fact, the potent  $\alpha_2$ -agonist dexmedetomidine, which has little affinity for imidazoline-specific binding sites (Ernsberger, unpublished data), induces profound sedation and near-anesthesia without lowering blood pressure (34,35). The nonimidazoline selective  $\alpha_2$ -agonist azepevole is more potent than clonidine in facilitating sedation and anesthesia (35) but is at least 100-fold less potent in inhibiting sympathetic activity (30,36).  $\alpha_2$ -Adrenergic receptors have also been implicated in the sedation produced by ethanol (37). Participation of imidazoline-specific mechanisms in ethanol-induced central nervous system depression has been ruled out (37).

#### IV. Evidence for Nonadrenergic Actions of Imidazolines

Thus far, we have reviewed evidence that imidazoline  $\alpha_2$ -agonists such as clonidine act specifically within the RVLM to lower blood pressure, that nonimidazoline  $\alpha_2$ -adrenergic agonists act specifically within the NTS to lower blood pressure and heart rate, and that  $\alpha_2$ -agonists act within the locus ceruleus and cerebral cortex to elicit central nervous system (CNS) depression. This difference in the site of action of different chemical classes of  $\alpha_2$ -agonists implies that an additional mechanism must be involved in the action of imidazoline  $\alpha_2$ -agonists within the RVLM. This additional mechanism would by definition be independent of the stimulation of  $\alpha_2$ -adrenergic receptors. In this section, we will review historical evidence for nonadrenergic actions of imidazolines and the characterization of  $I_1$ -imidazoline binding sites, which provide a potential molecular mechanism for the nonadrenergic actions of imidazolines.

Clonidine is a potent drug with multiple physiological effects (23,38). Early doubts that these diverse actions were entirely due to activation of  $\alpha_2$ -adrenergic receptors were raised when it was found that clonidine is a weak partial agonist

or even an antagonist in many  $\alpha_2$ -receptor systems (23). Furthermore, it was noted that  $\alpha$ -antagonists as well as  $\alpha$ -agonists lower blood pressure when administered into brainstem ventricles (39–41). For example, phentolamine, an imidazoline that acts as an antagonist at  $\alpha_1$ - and  $\alpha_2$ -adrenergic receptors, elicits a centrally mediated fall in blood pressure by inhibiting sympathetic outflow (40–43). The effect of clonidine is additive (43) or somewhat less than additive (42) with phentolamine, in contrast to the antagonism observed at  $\alpha_2$ -adrenergic receptors. Phentolamine also lowered blood pressure (by 14 mm Hg) upon microinjection into the RVLM of normotensive rats (44).

In the 1970s, Karppanen noted interactions between clonidine and imidazole compounds such as cimetidine and imidazole-4-acetic acid (IAA) and proposed that clonidine may act via brainstem "imidazole receptors" (45–47). The first direct evidence for a nonadrenergic action of imidazolines was reported by Bousquet and colleagues, who used microinjection to administer clonidine analogs directly into their site of action in the rostral ventrolateral medulla (RVLM) (18,48). Cirazoline, an imidazoline that is an  $\alpha_2$ -adrenergic antagonist, showed a clonidine-like vasodepressor action (48). In contrast,  $\alpha$ -methylnorepinephrine, a potent  $\alpha_2$ -agonist that lacks an imidazole ring, had no effect on blood pressure. These findings led to the proposal that the vasodepressor action of clonidine within the RVLM was mediated not by  $\alpha_2$ -adrenergic receptors but by a novel receptor specific for imidazolines.

A major line of evidence supporting the assumption that clonidine acted specifically through  $\alpha_2$ -adrenergic receptors was that [ $^3$ H]clonidine specifically labeled  $\alpha_2$ -adrenergic receptors in brain and platelet membranes (23). However, [ $^3$ H]clonidine binding assays in brain had always used cerebral cortex membranes, due to its very high density of  $\alpha_2$ -adrenergic receptors (23). When [ $^3$ H]clonidine binding assays were conducted using membranes from the RVLM, it was found that [ $^3$ H]clonidine labeled not only  $\alpha_2$ -adrenergic receptors but also a novel population of nonadrenergic binding sites that were insensitive to catecholamines and other adrenergic agents with a phenylethylamine structure and were specific for imidazoles and imidazolines (2,5,49–57).  $I_1$ -Imidazoline binding sites have been described in the medulla oblongata of cow (5,50,51,58,59), rat (54,60), and human (58). These sites have also been characterized in the peripheral nervous system, including adrenomedullary chromaffin cells and the carotid body (59,61), and in clonal neuron-like cell lines such as NG108-15 neuroblastoma-glioma (62) and PC12 pheochromocytoma cells (63). Primary cultures of astrocytes lack these imidazoline sites, although they express a moderate density of  $\alpha_2$ -adrenergic receptors (64). The lack of  $I_1$ -imidazoline sites in glia implies that, within the brain, they are predominantly neuronal.

$I_1$ -Imidazoline binding sites are distinct not only from adrenergic and histaminergic receptors (50,51) but also from imidazoline recognition sites labeled with [ $^3$ H]idazoxan. Imidazoline sites labeled by [ $^3$ H]idazoxan show 100-fold lower affinity for clonidine and do not recognize imidazoles such as cimetidine,

which have a moderately high affinity for I<sub>1</sub>-imidazoline sites (65–68). Unlike the nonadrenergic sites labeled by [<sup>3</sup>H]clonidine, [<sup>3</sup>H]idazoxan sites show high affinity for guanidines and thus have been termed “imidazoline-guanadinium receptive sites” (65–67,69). We have proposed the terminology of I<sub>1</sub>- and I<sub>2</sub>-imidazoline sites for the binding sites labeled by [<sup>3</sup>H]clonidine and [<sup>3</sup>H]idazoxan, respectively (52,54,70), and these terms were recently adopted by consensus (56).

The binding of [<sup>3</sup>H]*p*-aminoclonidine and [<sup>3</sup>H]clonidine to I<sub>1</sub>-imidazoline sites shows properties characteristic of known neurotransmitter receptors, since radioligand binding is rapid, specific, saturable, reversible, and of high affinity (5,50,51,54,58–60). Furthermore, the pharmacological profile of these binding sites is distinct from that of any known receptor. Although many of the compounds that bind to I<sub>1</sub>-imidazoline sites also bind to α<sub>1</sub>- or α<sub>2</sub>-adrenergic receptors, I<sub>1</sub>-sites fail to bind many other agents active at these receptors, including phenylethylamine agonists and the nonimidazoline antagonists such as phenoxybenzamine and SK&F86466. Similarly, although I<sub>1</sub>-imidazoline sites bind cimetidine and other agents active at histamine H<sub>2</sub> receptors, I<sub>1</sub>-sites fail to bind histamine agonists and antagonists lacking an imidazole ring, such as dimaprit, ranitidine, tiotidine, lupitidine, methapyraline, and thiazolyethylamine (Ernsberger, unpublished data). Further evidence that I<sub>1</sub>-imidazoline sites are distinct from histamine receptors is that there are no histamine-containing neurons or cell bodies in the RVLM (71,72), despite the presence of a high density of I<sub>1</sub>-imidazoline sites.

The heterogeneity of α<sub>2</sub>-adrenergic receptors has recently been established by radioligand binding and molecular cloning (73–76). Of the four or more proposed subtypes, the best known are α<sub>2A</sub>- and α<sub>2B</sub>-adrenergic receptors, which can be distinguished on the basis of the relative potencies of oxymetazoline, which is α<sub>2A</sub>-selective, and prazosin, which is α<sub>2B</sub>-selective (73,76). Prazosin also has high affinity for α<sub>2C</sub> receptors. The α<sub>2A</sub>-receptor predominates in most regions of the brain (73,77), including the NTS and the RVLM (78). The α<sub>2C</sub> subtype is mainly found in cerebral cortex (78). Consistent with the predominance of the α<sub>2A</sub> subtype, we noted very low affinity of prazosin at α<sub>2</sub>-adrenergic receptor binding sites in the bovine RVLM. Because all of the α<sub>2</sub>-adrenergic receptor subtypes show high affinity for epinephrine, SK&F 86466, and other nonimidazoline agonists and antagonists, whereas I<sub>1</sub>-imidazoline sites show micromolar or lower affinity for these agents, it is clear that I<sub>1</sub>-imidazoline sites do not represent another subtype of α<sub>2</sub>-adrenergic receptors but are a distinct class of putative membrane receptors.

The endogenous ligand for imidazoline receptors is unknown. The catecholamines can be excluded, since they do not bind to these sites. Histamine is an imidazole neurotransmitter, but the ligand specificity of imidazoline receptors is distinct from that of histamine H<sub>1</sub>, H<sub>2</sub>, or H<sub>3</sub> receptors. The probable endogenous ligand at imidazoline receptors is a clonidine-displacing sub-

stance (CDS) isolated from the brain and originally described as an endogenous α-agonist (79). CDS is a competitive inhibitor at both imidazoline and α<sub>2</sub>-adrenergic sites, but exhibits 30-fold higher affinity for imidazoline sites (51,62). CDS may be structurally related to clonidine, since it cross-reacts with anti-clonidine antibodies (80). Several laboratories have major initiatives dedicated to purifying and identifying this substance (56).

Independent replication of many of the key findings supporting the existence of I<sub>1</sub>-imidazoline receptors have been reported by laboratories on four continents. For example, a recent article replicates the basic findings characterizing imidazoline receptors in bovine RVLM and extends these results to the human RVLM (58). Other reports characterize binding sites in rat brain (60), human platelets (81) and bovine adrenal medulla (82) that appear indistinguishable from I<sub>1</sub>-imidazoline sites of the bovine RVLM.

### V. Evidence That an I<sub>1</sub>-Imidazoline Receptor in the RVLM Mediates a Vasodepressor Response

The primary findings supporting a role of I<sub>1</sub>-imidazoline receptors in the action of imidazoline vasodepressor agents within the RVLM are summarized in Table 1. An essential first criterion, the presence of I<sub>1</sub>-imidazoline sites in the RVLM, is well established. The vasodepressor action of cirazoline (48) and phentolamine, two imidazolines which are α<sub>2</sub>-antagonists, is difficult to reconcile with the hypothesis that all of the actions of imidazolines are mediated by adrenergic mechanisms. Participation of α<sub>1</sub>-adrenergic receptors in the effects of cirazoline and phentolamine is unlikely, since the α<sub>1</sub>-agonist methoxamine and the α<sub>1</sub>-antagonist prazosin had no effect on blood pressure following microinjection into the cat RVLM (83,84). This implies a nonadrenergic action of imidazolines in the RVLM.

Further evidence against a purely α<sub>2</sub>-adrenergic mechanism in the RVLM is the finding that phenylethylamine α-agonists are relatively weak in eliciting vasodepression in the RVLM relative to imidazolines, despite the fact that phenylethylamines, including the catecholamines epinephrine and norepinephrine, are full agonists whereas the imidazolines are only partial agonists and therefore can act as antagonists in the presence of an endogenous agonists. α-Methylnorepinephrine, a potent full agonist at α<sub>2</sub>-adrenergic receptors, had no significant effect on blood pressure at doses up to 40 nmol when microinjected into the cat RVLM (48). Follow-up studies showed that epinephrine, norepinephrine, and phenylephrine were also completely ineffective up the same high dose (83). Comparable experiments in the rat have yielded inconsistent results. In one study in spontaneously hypertensive rats, α-methylnorepinephrine was completely ineffective microinjected into the RVLM, despite the robust response to clonidine in the same region (85). α-Methylnorepinephrine did elicit a moderate hypotension when administered into the nearby parapyramidal regions

**Table 1** Evidence Supporting a Role for I<sub>1</sub>-Imidazoline Receptors in Vasodepressor Actions in the RVLM

1. I<sub>1</sub>-Imidazoline binding sites are localized to the RVLM, as shown by membrane binding studies (5,50,51,57,91) and by autoradiographic visualization (91,121) (Fig. 5).
2. Imidazolines that are antagonists at  $\alpha_2$ -adrenergic receptors can elicit vasodepressor responses within the RVLM, including cirazoline (48) and phentolamine (40-44).
3.  $\alpha_2$ -Adrenergic antagonists fail to increase blood pressure when administered into the RVLM and may actually decrease pressure (5,84,87,100) (Figs. 6 and 9), implying that  $\alpha_2$ -adrenergic receptors in the RVLM are not activated under resting conditions. In contrast, the I<sub>1</sub>/ $\alpha_2$  antagonist efaroxan raises pressure when administered by itself into the RVLM (87) (Fig. 9), implying that I<sub>1</sub>-imidazoline receptors are activated under resting conditions.
4.  $\alpha_2$ -Adrenergic full agonists having a phenylethylamine or guanidine structure are either completely inactive (48,83,85,86) or relatively weak (5,44) (Fig. 1) in eliciting vasodespression in the RVLM.
5. Upon microinjection into the NTS, phenylethylamine full  $\alpha_2$ -agonists are extremely potent in eliciting vasodepression (1,18-21) (Fig. 1), whereas imidazoline partial  $\alpha_2$ -agonists, including clonidine, are very weak (11,20,22) (Fig. 2). Unlike the RVLM, microinjection of  $\alpha_2$ -antagonists into the NTS elicit elevations in blood pressure. The pharmacological profile of vasodepressor responses in the NTS closely corresponds to an  $\alpha_2$ -adrenergic receptor type and is clearly distinct from the pharmacological profile in the RVLM.
6. Second-generation central antihypertensives such as rilmenidine and moxonidine have reduced affinity at  $\alpha_2$ -adrenergic receptors and attenuated sedative side effects, but they retain effectiveness in lowering blood pressure (2,53,90,91).
7. Binding affinity at I<sub>1</sub>-imidazoline sites predicts clinical antihypertensive efficacy, whereas  $\alpha_2$ -adrenergic binding affinity or  $\alpha_2$ -efficacy are unrelated (57) (Figs. 3 and 4).
8. Binding affinity at I<sub>1</sub>-imidazoline sites predicts vasodepressor activity within the RVLM, whereas  $\alpha_2$ -adrenergic binding affinity or  $\alpha_2$ -efficacy are unrelated (5,90).
9. Cimetidine, which has no affinity for  $\alpha_2$ -adrenergic receptors, elicits vasodepression when microinjected into the RVLM with a potency consistent with its affinity at I<sub>1</sub>-imidazoline sites (5).
10. The vasodepressor actions of imidazolines (clonidine, rilmenidine, or moxonidine) given either intravenously or by RVLM microinjection, can be prevented or reversed by RVLM microinjection of antagonists with high affinity for I<sub>1</sub>-imidazoline sites (idazoxan, efaroxan), whereas nonimidazoline antagonists (SK&F 86466, yohimbine, rauwolscine) have little effect (2,5,9,87,100) (Figs. 5, 6, and 9).

containing the B3 serotonergic neurons (85). A study in normotensive rats found modest (10 to 18 mm Hg) vasodepressor responses to bilateral microinjection of 1 nmol of epinephrine, norepinephrine,  $\alpha$ -methylnorepinephrine, and guanabenz (5). Another study found guanabenz to be inactive when applied to the ventral surface of the brainstem, in contrast to the potent hypotensive effect of clonidine

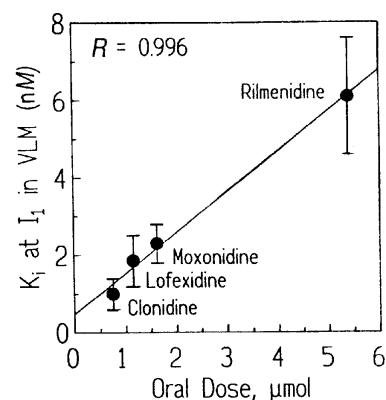
applied in this manner (86). A single study found  $\alpha$ -methylnorepinephrine to lower blood pressure and heart rate effectively in the RVLM (44). However, the doses required are 30-fold higher than those needed to elicit blood pressure and heart rate responses in the NTS (20) (Fig. 1). Because multiple injections were tested in each rat in the study reporting large responses to  $\alpha$ -methylnorepinephrine in rats (44), and because the micropipette must pass through the NTS to target the RVLM, minor seepage along the micropipette tract (3% of total volume) could account for the effect observed.

There are major differences in pharmacological activity of  $\alpha$ -adrenergic agonists between the NTS and the RVLM, with phenylethylamines such as  $\alpha$ -methylnorepinephrine much more potent in the NTS and imidazolines such as clonidine being much more potent in the RVLM (Figs. 1 and 2). The contrast between NTS and RVLM is even greater for epinephrine, which is active in the NTS after unilateral injection of 0.02 nmol (20), whereas 50-fold higher doses are required bilaterally within the RVLM to elicit a comparable response (5). Oxymetazoline is completely inactive in the NTS at doses up to 20 nmol (20), whereas in the RVLM oxymetazoline is nearly as potent as clonidine (5). The relative effectiveness of different  $\alpha$ -agonists in the NTS is fully consistent with an  $\alpha_2$ -adrenergic receptor mechanism, with an order of potency of epinephrine > norepinephrine >  $\alpha$ -methylnorepinephrine and with reduced effectiveness of partial agonists such as clonidine and oxymetazoline.  $\alpha_2$ -Antagonists also have contrasting effects in the NTS and RVLM. When microinjected into the NTS, yohimbine even in very low doses (<1 nmol) induces prolonged increases in blood pressure (19,21,22,25). In contrast, yohimbine and idazoxan had no effect in low doses and actually lowered pressure when microinjected into the cat RVLM (84), and SK&F 86466 induced a dose-dependent decrease in blood pressure in normotensive rats (5) and in hypertensive rats (87). The  $\alpha_2$ -adrenergic hypothesis of central vasodepressor action cannot account for the differences between RVLM and NTS sites of injection. Furthermore, the failure of  $\alpha_2$ -adrenergic antagonists to elevate pressure when applied within the RVLM implies that there is not a tonically active depressor system coupled to  $\alpha_2$ -receptors in this region, in contrast to a highly active  $\alpha_2$ -adrenergic receptor system of the NTS.

One might argue that the catecholamines are relatively weak when microinjected into the RVLM because they are subject to uptake and degradation, whereas the imidazolines are not. However, this cannot account for the high potency of these agents upon microinjection into the NTS, an area with high concentration of noradrenergic terminals containing uptake sites and degradative enzymes for catecholamines. Myelinated axons make up the bulk of the brain tissue within the RVLM and only a low density of neurons is present relative to the NTS (88,89). The primary effect of uptake and degradation appears to be upon duration of action. In the NTS,  $\alpha$ -methylnorepinephrine has a longer duration of action relative to norepinephrine (20) and is less susceptible to de-

gradation. Nonetheless, norepinephrine is more potent than  $\alpha$ -methylnorepinephrine in lowering blood pressure. In all probability, the high concentrations of catecholamine applied by microinjection ( $>1$  mM in most cases) greatly exceed the capacity of local uptake and degradation mechanisms within the small ( $>1$  mm<sup>3</sup>) sphere of tissue affected by the injection.

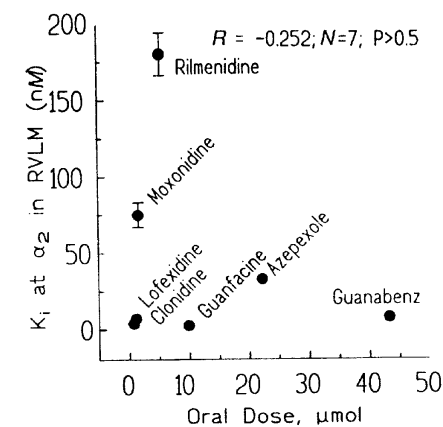
The clinical application of I<sub>1</sub>-imidazoline receptor pharmacology has been realized with the introduction of two selective I<sub>1</sub>-agonists for the treatment of essential hypertension. Rilmenidine, now on the market in France, and moxonidine, now available in Germany and soon to be introduced throughout Europe, represent a second generation of centrally acting antihypertensive agents that cause greatly diminished side effects of sedation and dry mouth relative to first-generation agents such as clonidine, guanfacine and guanabenz (2,53,90,91). The reduced side effects from rilmenidine and moxonidine appear to be due to lower  $\alpha_2$ -adrenergic affinity. Controlled studies in laboratory animals also support the contention that rilmenidine and moxonidine have reduced sedative side effects. As reviewed in Section III above, the locus ceruleus is a likely site for the sedative action of  $\alpha_2$ -agonists. Rilmenidine is 60-fold less potent than clonidine in inhibiting the activity of locus ceruleus neurons, whether given intravenously or by iontophoresis (92). Rilmenidine does not prolong barbiturate-induced sleeping time in rats and can antagonize the sedative action of clonidine (93). Rilmenidine was 80-fold less potent than clonidine in reducing locomotor activity in rats (94). Moxonidine was over 20-fold less potent than clonidine in a locomotor activity test in mice (95).



**Figure 3** Relationship between affinity at I<sub>1</sub>-imidazoline sites in the bovine VLM and therapeutic potency in hypertensive humans. Clinical doses were estimated from long-term clinical trials reporting effective control of human essential hypertension. The affinity of each agent at I<sub>1</sub>-imidazoline binding sites in the VLM is highly correlated with clinically effective dose ( $P < 0.01$ ). The four compounds are similar in their chemical properties (hydrophobicity, pK<sub>a</sub>) and their pharmacokinetics. (From Ref. 57.)

We compared binding affinity at I<sub>1</sub>-imidazoline sites to the dose required to treat human essential hypertension for the four imidazoline compounds that have been subjected to controlled clinical trials (57). As shown in Figure 3, the effective dose for control of essential hypertension is tightly correlated with binding affinity at bovine RVLM I<sub>1</sub>-imidazoline sites. Rilmenidine has a lower affinity for imidazoline sites in the RVLM than clonidine (2,96,97), which is consistent with its reduced potency clinically. Lofexidine and moxonidine are intermediate with respect to both binding affinity and clinical efficacy. The slope of this correlation is very close to one ( $1.05 \pm 0.07$ ), implying a one-to-one correspondence of binding affinity and clinical potency. This correlation implicates the putative I<sub>1</sub>-imidazoline receptor in the therapeutic actions of these agents and also implies that there are few species differences in the characteristics of I<sub>1</sub>-imidazoline sites, in agreement with a direct comparison of RVLM I<sub>1</sub>-imidazoline sites with those of human platelets (152).

We compared binding affinity at  $\alpha_2$ -adrenergic sites to the clinically effective dose for seven compounds. Dosages for six of the compounds were from clinical trials (57), whereas azepexole data were from a dosing study in normotensive humans (98). Unlike I<sub>1</sub>-imidazoline affinity, the  $\alpha_2$ -affinity of central antihypertensive agents was not correlated with clinical efficacy, as shown in Figure 4. This result is difficult to reconcile with the currently accepted notion that  $\alpha_2$ -adrenergic receptors alone are responsible for central antihypertensive actions. It might be argued that differences in blood-brain-barrier penetration or pharmacokinetics might obscure a relationship between  $\alpha_2$  potency and effi-



**Figure 4** Relationship between affinity at  $\alpha_2$ -adrenergic receptor sites in the VLM and clinical potency. Error bars are not visible for four of the compounds because the standard error was smaller than the size of the symbol. Other details as in Figure 3. (Modification of an original figure in Ref. 57.)

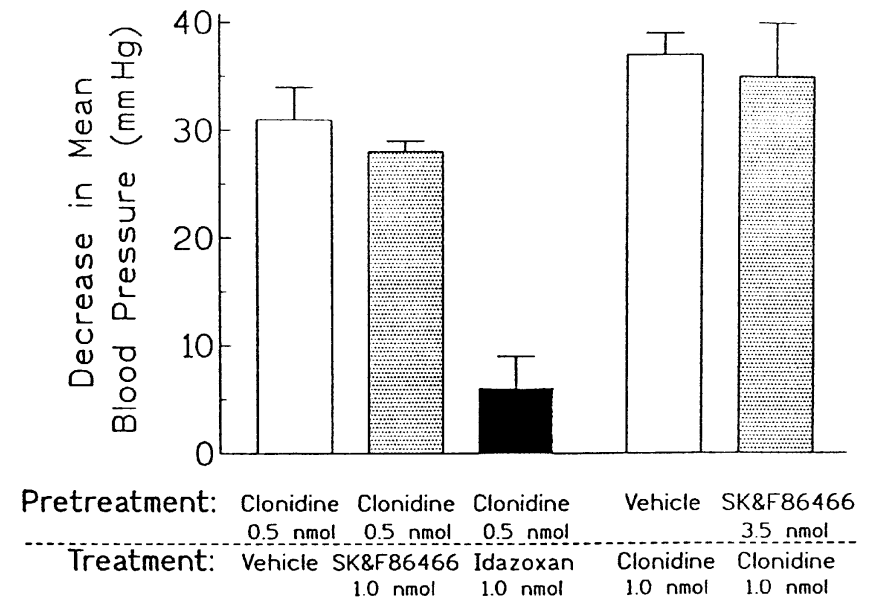
cacy. However, all seven of the comparison agents readily gain access to the central nervous system (57). In fact, of the seven compounds, guanfacine shows the best penetration of the blood-brain barrier, the highest accumulation within the central nervous system, and the longest half-life (21 h), yet it is 10-fold less potent than clonidine despite 10-fold higher affinity at  $\alpha_2$ -adrenergic receptors (26,27). Furthermore, the  $\alpha_2$ -affinity of the centrally acting antihypertensives varied over an almost 100-fold range, in contrast to the 6-fold range of  $I_1$ -affinities for this class of agent. This result implies that a high affinity at  $I_1$ -imidazoline receptors (1 to 6 nM) may be a property that distinguishes potent centrally acting antihypertensive agents.

A correlation between vasodepressor actions and  $I_1$ -imidazoline binding affinity has been reported for controlled laboratory studies as well as for clinical antihypertensive potency data. The ability of a series of imidazoline, imidazole, and phenylethylamine agents to lower blood pressure and heart rate when microinjected into the RVLM of normotensive rats was correlated with binding affinity at  $I_1$  imidazoline receptors (5,90). In the original series of six compounds, there were significant correlations of  $I_1$ -imidazoline affinity with vasodepressor and bradycardiac responses ( $r=0.84$  and  $0.89$ , respectively) (5). Addition of moxonidine to make a total of seven compounds maintained the correlation with vasodepressor response ( $r=0.93$ ,  $P=0.0025$ ) (90). The further addition of rilmenidine to the series decreased the correlation slightly ( $r=0.79$ ,  $P=0.02$ ), owing to rilmenidine's low potency compared to that predicted from its  $I_1$  affinity. The potency of rilmenidine in humans, however, closely corresponds to the value predicted from its  $I_1$ -imidazoline affinity (see Fig. 3), suggesting that the low efficacy of rilmenidine in the RVLM of rats is a peculiarity of that species.

In contrast to the strong correlation of cardiovascular actions with  $I_1$ -imidazoline affinity,  $\alpha_2$ -adrenergic affinity or efficacy is unrelated to functional potency upon administration into the RVLM. The correlation of  $\alpha_2$ -affinity and vasodepressor responses was not significant for the original series of 11  $\alpha_2$ -agonists ( $r=-0.05$ ) (5) or with the addition of moxonidine and rilmenidine ( $r=-0.17$ ) (90). In fact, there was a nonsignificant tendency for compounds with the lowest affinity at  $\alpha_2$ -adrenergic receptors to have the greatest effect on blood pressure.  $\alpha_2$ -Affinity was also unrelated to bradycardiac potency ( $r=0.10$ ) (5). There was also no relationship between intrinsic efficacy at  $\alpha_2$ -adrenergic receptors and cardiovascular effects in the RVLM, because partial agonists such as clonidine and oxymetazoline were more effective than full agonists such as epinephrine, norepinephrine, and  $\alpha$ -methylnorepinephrine.

The one-to-one correspondence of binding affinity and both clinical and experimental potency is an important finding, because it fulfills a major criterion for the identification of a binding site as a functional receptor. On the basis of these data, the Nomenclature Committee of the International Union of Pharmacology has accepted the terminology  *$I_1$ -imidazoline receptor* for inclusion in the 1994 handbook of receptor nomenclature (*Trends in Pharmaco-*

*logical Sciences Supplement*, 1994). However, these correlational data are not direct tests of the relative participation of  $I_1$ -imidazoline and  $\alpha_2$ -adrenergic receptors in the vasodepressor response of imidazolines mediated within the RVLM. We have used selective antagonists in an attempt to separate the relative roles of these receptor types. In one study, clonidine (0.5 nmol) was microinjected into the RVLM, followed 4 min later by administration via the same route of vehicle or antagonist (5). Some 6 min after the second microinjection, rats receiving vehicle showed a maintained vasodepressor response of over 30 mm Hg (Fig. 5; open bar). The selective  $\alpha_2$ -antagonist SK&F 86466 (1 nmol) did not reverse the effect of clonidine (stippled bar). However, idazoxan (1 nmol), an  $\alpha_2$ -antagonist with a high affinity for  $I_1$ -imidazoline receptors was able to completely and rapidly reverse the effect of clonidine (solid bar). Because both SK&F 86466 and idazoxan are potent  $\alpha_2$ -antagonists, the major difference be-

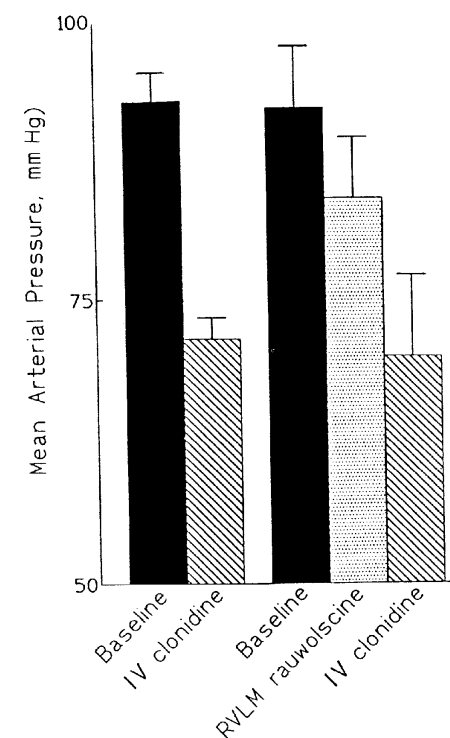


**Figure 5** Effect of imidazoline and nonimidazoline  $\alpha_2$ -adrenergic antagonists on the vasodepressor response to microinjection of clonidine into the RVLM. Data were re-analyzed from Ernsberger and colleagues (5). In this series of experiments, two microinjections were made. In the first experiment, clonidine was microinjected first (0.5 nmol) followed 4 min later by either normal saline vehicle (open bar), the selective  $\alpha_2$ -antagonist SK&F 86466 (1.0 nmol; stippled bar), or the  $\alpha_2/I_1$ -antagonist idazoxan (1.0 nmol; solid bar). In the second experiment, a higher dose of SK&F 86466 was tested. Pretreatment by microinjection of vehicle (open bar) or SK&F 86466 (3.5 nmol) was followed by microinjection of clonidine (1.0 nmol). Idazoxan completely reversed the effect of clonidine, whereas SK&F 86466 neither reversed nor prevented the action of clonidine, even when given at a dose 3.5-fold higher than that used for idazoxan.

tween them is the higher affinity of idazoxan for  $I_1$ -imidazoline sites. Thus, it appears that  $I_1$ -imidazoline receptors mediate most if not all of the action of clonidine within the RVLM. To test for the possibility that 1 nmol of SK&F 86466 was insufficient to achieve full blockade of  $\alpha_2$ -adrenergic receptors, the selective  $\alpha_2$ -antagonist was administered at a dose of 3.5 nmol. SK&F 86466 rapidly lowered blood pressure by  $27 \pm 2$  mm Hg and microinjection of clonidine (1 nmol) lowered blood pressure an additional  $35 \pm 5$  mm Hg, which did not differ from the effect of clonidine following pretreatment with vehicle ( $37 \pm 2$  mm Hg) (5) (Fig. 5). The fact that  $\alpha_2$ -antagonists lower blood pressure when microinjected into the RVLM and that the action of clonidine is additive with that of an  $\alpha_2$ -antagonist further argues against a primary role of  $\alpha_2$ -adrenergic receptors in control of cardiovascular function by the RVLM.

Initial tests of the effects of selective antagonists on the action of imidazolines within the RVLM have used microinjection for the delivery of both agonist and antagonist. However, local microinjection introduces high local concentrations of the drug, whose effects may not be entirely representative of the actions of systemically administered agents. Furthermore, in clinical practice, centrally acting drugs are distributed throughout the organism and not confined to a specific nucleus. Therefore, in some recent studies, we have examined the ability of locally injected antagonists within the RVLM to prevent the effect of systemically administered drug. In addition, we have used second-generation central antihypertensive agents with selectivity for  $I_1$ -imidazoline sites relative to  $\alpha_2$ -adrenergic receptors. Microinjection of the selective  $\alpha_2$ -antagonist SK&F 86466 into the RVLM in either a low dose (1 nmol) or a high dose (10 nmol) did not significantly attenuate the effect of intravenous rilmenidine (0.5 mg/kg). In contrast, the  $\alpha_2/I_1$  antagonist idazoxan completely abolished the effect of intravenous rilmenidine even at a low dose (1 nmol) (2). More recently, we have shown that microinjection of the selective  $I_1$ -imidazoline antagonist efuroxan (10 nmol) into the RVLM completely prevented the hypotensive action of intravenous moxonidine (40  $\mu$ g/kg) and appeared to unmask a direct vasoconstrictor effect on the vasculature (87). These studies implicate  $I_1$ -imidazoline receptors within the RVLM in the action of systemically administered imidazolines and confirm the RVLM as the site of action of second-generation central antihypertensives as well as the prototype drug clonidine.

Accumulating evidence supports the role of  $I_1$ -imidazoline receptors in vasodepressor actions of imidazolines. Intracisternal administration of a high dose of rauwolscine (300 nmol) completely abolished the action of the  $\alpha_2$ -selective agonist S 8350 (30 nmol) but only attenuated the effect of clonidine (3 nmol) by one-third (99). Elegant support for a role of nonadrenergic mechanisms in the action of clonidine was provided by Nosjean and Guyenet (100). Intravenous clonidine (5  $\mu$ g/kg) produced a significant fall in blood pressure relative to baseline (Fig. 6). Microinjection of rauwolscine (2 nmol) into the RVLM, like other  $\alpha_2$ -antagonists, elicited a fall in blood pressure (see above). Subsequent injection



**Figure 6** Vasodepressor action of intravenous clonidine (20  $\mu$ g/kg) with or without blockade of  $\alpha_2$ -adrenergic receptors in the RVLM by local microinjection of rauwolscine (16 nmol). (Data are plotted from Ref. 100.) The fall in mean arterial pressure elicited by clonidine was not affected by pretreatment with rauwolscine in the RVLM. Rauwolscine pretreatment did, however, block the effect of the serotonin 5-HT<sub>1a</sub> agonist 8-OH-DPAT (not shown). A much lower dose of the imidazoline antagonist idazoxan (2 nmol) microinjected into the RVLM completely blocked the effect of intravenous clonidine (not shown).

tion of clonidine produced a depressor response indistinguishable from the control response. Rauwolscine was able to block the responses to systemic 8-hydroxy-DPAT, a serotonin 5-HT<sub>1a</sub> agonist, demonstrating that rauwolscine is an effective antagonist for 5-HT<sub>1a</sub>-serotonergic as well as  $\alpha_2$ -adrenergic receptors. In contrast to rauwolscine, idazoxan completely blocked the action of clonidine after microinjection into the RVLM (100) (data not shown).

Much of the support for a role of  $I_1$ -imidazoline receptors in the action of clonidine comes from studies in anesthetized rats. However, one study of fourth-ventricle administration of drugs in conscious, freely moving rats showed that the selective  $\alpha_2$ -agonist BHT 920 actually increased blood pressure and plasma catecholamine levels, whereas the  $\alpha_2/I_1$  agonist clonidine lowered blood pressure and catecholamines (101). The  $\alpha_2$ -antagonist rauwolscine (10  $\mu$ g) did not

abolish the action of clonidine (1.0  $\mu\text{g}$ ), ruling out a role of  $\alpha_2$ -adrenergic receptors (101). An independent study using intracisternal application in conscious spontaneously hypertensive (SHR) rats determined dose-response curves for a series of imidazoline compounds with similar  $\alpha_2$ -affinity but varying affinity for  $I_1$ -imidazoline sites. Vasodepressor potency as defined by  $\text{ED}_{50}$  values was correlated with  $I_1$  but not  $\alpha_2$  affinity (102). These findings imply that the role of  $I_1$ -imidazoline receptors in cardiovascular regulation may be more pronounced in conscious animals.

In vivo electrochemical studies support a role for nonadrenergic receptors in the actions of imidazolines. Intravenous clonidine or rilmenidine inhibited catecholamine activity in the RVLM in parallel with a fall in blood pressure (9,30). Catecholaminergic activity in the locus ceruleus was also inhibited, but higher doses of the selective  $I_1$ -agonist rilmenidine were required to inhibit the locus coeruleus relative to the RVLM (30). Intracisternal administration of the  $\alpha_2/I_1$  antagonist idazoxan (5 nmol) completely prevented the fall of both the blood pressure and catecholamine activity. In contrast, the  $\alpha_2/5\text{-HT}$  antagonist yohimbine (5 nmol) did not alter either effect of clonidine (9). The superiority of idazoxan was not due to more effective  $\alpha_2$ -blockade, because catecholamine activity in the locus ceruleus was reversed by yohimbine but not idazoxan, showing that yohimbine is actually more effective in antagonizing a true  $\alpha_2$ -adrenergic effect. These data support the idea that there is a mechanism in the RVLM which is selectively affected by imidazoline agonists and antagonists and shows a distinct specificity relative to the classical  $\alpha_2$ -adrenergic receptor system expressed in the locus ceruleus.

The ability of clonidine, a mixed  $I_1/\alpha_2$  agonist, to elicit parallel decreases in blood pressure and RVLM catecholamine activity (9) is not observed for dexmedetomidine, a specific  $\alpha_2$ -agonist (103). Unlike clonidine, which transiently elevates blood pressure but then produces prolonged hypotension, dexmedetomidine raises blood pressure persistently when given intravenously because the direct vasoconstrictor action on vascular  $\alpha_2$ -adrenergic receptors overwhelms any centrally mediated fall in blood pressure. This is not due to a failure to penetrate the brain, because dexmedetomidine enters the CNS readily and elicits profound sedation at very low doses (35). When administered intracerebroventricularly to anesthetized rats, dexmedetomidine only transiently lowers blood pressure. In contrast, catecholamine activity in the RVLM is profoundly depressed, falling to one-third of baseline within 45 min (103). Mean blood pressure measured simultaneously was decreased less than 10 mm Hg. Thus, in contrast to the correlated and parallel decreases in blood pressure and RVLM catecholamine activity elicited by the imidazolines clonidine and rilmenidine, the selective  $\alpha_2$ -agonist dexmedetomidine decreases catecholamine activity to a much greater extent than it decreases blood pressure. The selective effect of  $\alpha_2$ -adrenergic agonists on catecholamine activity is probably due to an  $\alpha_2$ -autoreceptor mechanism present on adrenergic neurons in the RVLM. These  $\alpha_2$ -autoreceptors might not be involved in controlling sympathetic outflow.

## VI. New Tests of the $I_1$ -Imidazoline Receptor Hypothesis

The discovery of selective agonists and antagonists binding with high affinity to  $I_1$ -imidazoline sites suggests new tools enabling direct tests of the hypothesis that  $I_1$ -imidazoline sites are functional receptors. We used these selective agents in autoradiographic studies to separately visualize  $\alpha_2$ -adrenergic and  $I_1$ -imidazoline binding sites in the medulla and for functional studies in medulla and carotid body in vivo. For autoradiography, slide-mounted brainstem sections were preincubated for 1 h, then incubated 1 h with 0.5 nM [ $^{125}\text{I}$ ]p-iodoclonidine on adjacent sections;  $I_1$  and  $\alpha_2$  were selectively blocked with 10  $\mu\text{M}$  epinephrine and moxonidine, respectively. Autoradiography of  $I_1$ -imidazoline and  $\alpha_2$ -receptors in rat medulla and their interaction with moxonidine is shown in Figure 7. Panel A shows total [ $^{125}\text{I}$ ]p-iodoclonidine binding in the presence of vehicle alone. Total binding of [ $^{125}\text{I}$ ]p-iodoclonidine is diffusely distributed throughout the medulla. Panel B shows an adjacent section incubated with 10  $\mu\text{M}$  epinephrine in order to mask  $\alpha_2$ -adrenergic sites. The horizontal arrow in Panel B indicates the NTS. Labeling in the NTS was almost completely eliminated by epinephrine, indicating that [ $^{125}\text{I}$ ]p-iodoclonidine labels mainly  $\alpha_2$ -adrenergic receptors in this dorsal region. The oblique arrow indicates the RVLM. Labeling in the RVLM was maintained in the presence of epinephrine, suggesting that a non-adrenergic site is mainly present in this region. Panel C shows another nearly adjacent section incubated with 100 nM moxonidine to mask  $I_1$ -imidazoline sites. The oblique arrow indicates the RVLM, which shows a loss of labeling in the presence of moxonidine, particularly on the left side of the section. The remaining labeling in the RVLM region presumably represents  $\alpha_2$ -adrenergic binding sites. Labeling in the dorsal medulla is preserved. Panel D shows an adjacent section incubated with 10  $\mu\text{M}$  BDF-6143 to mask both  $I_1$  and  $\alpha_2$  sites. The remaining labeling is defined as nonspecific.

These autoradiograms imply that  $I_1$ -imidazoline sites, defined as [ $^{125}\text{I}$ ]p-iodoclonidine binding sites labeled in the presence of epinephrine to block  $\alpha_2$  sites, are absent in the NTS but are widely distributed across reticular areas of the medulla, including the RVLM. A similar pattern was observed in the pons (not shown). The locus ceruleus in the dorsal pons expressed mainly  $\alpha_2$ , while  $I_1$  were present in A5 and ventral tegmental areas. We conclude that  $I_1$ -imidazoline sites are localized to cardiorespiratory regions of the reticular formation.

Direct microinjection of substances into brain tissue is powerful technique for testing the sites and mechanism of action of CNS drugs. However, in most studies, interpretation of the results is severely limited by a lack of knowledge of the brain region receiving the drug by diffusion. To address this issue, we characterized the pharmacokinetics of [ $^3\text{H}$ ]moxonidine microinjected into the RVLM. Microinjection of 4 nmol [ $^3\text{H}$ ]moxonidine elicited a typical vaso-depressor response at 8 min after injection (not shown). The distribution of [ $^3\text{H}$ ]moxonidine in the rostral-caudal plane was determined by scintillation counting of alternate transverse sections through the medulla. As shown in