



Provided by the author(s) and University of Galway in accordance with publisher policies. Please cite the published version when available.

Title	Modulation of stress by imidazoline binding sites: Implications for psychiatric disorders
Author(s)	Smith, Karen L.; Jessop, David S.; Finn, David P.
Publication Date	2009-07-07
Publication Information	Smith, Karen L., Jessop, David S., & Finn, David P. (2009). Modulation of stress by imidazoline binding sites: Implications for psychiatric disorders. <i>Stress</i> , 12(2), 97-114. doi: 10.1080/10253890802302908
Publisher	Taylor & Francis
Link to publisher's version	https://doi.org/10.1080/10253890802302908
Item record	http://hdl.handle.net/10379/15080
DOI	http://dx.doi.org/10.1080/10253890802302908

Downloaded 2024-04-27T01:13:47Z

Some rights reserved. For more information, please see the item record link above.



REVIEW

Modulation of stress by imidazoline binding sites: implications for psychiatric disorders

¹Karen L. Smith, ²David S. Jessop, ¹David P. Finn

¹Department of Pharmacology and Therapeutics, NCBES Neuroscience Cluster, National University of Ireland, Galway, University Road, Galway, Ireland; ²Henry Wellcome Laboratories for Integrative Neuroscience and Endocrinology, University of Bristol, Bristol, BS1 3NY, UK

CORRESPONDING AUTHOR:

Dr. David P. Finn,

Department of Pharmacology and Therapeutics,

National University of Ireland, Galway,

University Road,

Galway,

Ireland.

Tel: +353 (0)91 495280

Fax: +353 (0)91 525700

Email: David.Finn@nuigalway.ie

Note to Editor-in-Chief: 60 manuscript pages, 3 figures & 2 tables

ABSTRACT

In this review we present evidence for the involvement of imidazoline binding sites (IBS) in modulating responses to stress, through central control of monoaminergic and hypothalamo-pituitary-adrenal (HPA) axis activity. Pharmacological and physiological evidence is presented for differential effects of different IBS subtypes on serotonergic and catecholaminergic pathways involved in control of basal and stress-stimulated HPA axis activity. IBS ligands can modulate behavioural and neuroendocrine responses in animal models of stress, depression and anxiety, and a body of evidence exists for alterations in central IBS expression in psychiatric patients, which can be normalised partially or fully by treatment with antidepressants. Dysfunction in monoaminergic systems and the HPA axis under basal and stress-induced activation has been extensively reported in psychiatric illnesses. On the basis of the literature, we suggest a potential therapeutic role for selective IBS ligands in the treatment of depression and anxiety disorders.

Keywords: anxiety, depression, hypothalamo-pituitary-adrenal axis, imidazoline binding sites, monoamines.

INTRODUCTION

Aversive or stressful experiences provoke an array of behavioural and physiological changes which facilitate an organism's ability to cope. In recent years, thinking has moved away from the stress response as a general response and towards the consensus that adaptive responses to a particular stressor are specific to the nature, duration and intensity of the stressor (Pacak, 2000; Sapolsky *et al.*, 2000; Pacak and Palkovits, 2001; McEwen, 2007). The initiation of an appropriately graded response to a particular stressor is of vital importance as inapt responses may lead to chronic stress complications with consequent immunosuppression, and predisposition to infections and illness (Chrousos and Gold, 1992; Sapolsky *et al.*, 2000). Inappropriate responses to stress may also affect personality development and behaviour (Heim *et al.*, 2004; Gollan *et al.*, 2005; Popma *et al.*, 2007) and increase susceptibility to both the onset and severity of mental illness, particularly anxiety disorders, depression (Finlay-Jones and Brown, 1981; Brown *et al.*, 1995; Kendler and Karkowski-Shuman, 1997; Kendler *et al.*, 1999) and schizophrenia (Lahniers and White, 1976; Gruen and Baron, 1984; Day *et al.*, 1987).

The two major physiological systems mediating the stress response are the sympathetic nervous system and the hypothalamo-pituitary-adrenal (HPA) axis. While there is some evidence for altered sympathetic nervous system activity in psychiatric disorders, little if any strong evidence exists to support a causal role of sympathetic nervous system dysfunction in stress-related psychiatric disorders. In contrast, there is now a significant body of evidence implicating dysfunction of the HPA axis in the aetiology of various psychiatric and stress-related disorders (Zacharko and Anisman, 1991; Connor *et al.*, 1997; Dubrovsky, 2000).

Depression and the HPA axis

Many studies have examined the association between depressive illness and HPA axis abnormalities (for recent review see Thomson and Craighead, 2007). There is evidence that the negative feedback loop regulated by glucocorticoid hormones is deficient in depressed patients and a feed-forward hyperactivation of the HPA axis is thought to occur (Holsboer, 2000; Parker *et al.*, 2003). Specifically, decreased corticosteroid receptor function, elevated plasma cortisol, an enhanced adrenal response to adrenocorticotrophic hormone (ACTH), a blunted pituitary ACTH response to corticotrophin-releasing factor (CRF) as well as adrenal and pituitary enlargement are commonly observed in patients with depression (Krishnan *et al.*, 1991; Rubin *et al.*, 1996; Modell *et al.*, 1997; Scott and Dinan, 1998; Arborelius *et al.*, 1999; Holsboer, 2000; Parker *et al.*, 2003). Additionally, it has recently been shown that increased ACTH secretion may occur in depressed patients regardless of cortisolemic status (for review see Bornstein *et al.*, 2008). Importantly, hyperactivation of the HPA axis is normalized by long-term antidepressant treatment (Greden *et al.*, 1983; De Bellis *et al.*, 1993; Michelson *et al.*, 1997; Pariante *et al.*, 2004). Interestingly, the failure of CRF hypersecretion to normalize subsequent to treatment is predictive of early relapse of depressive symptoms (Greden *et al.*, 1983; Banki *et al.*, 1992; Ribeiro *et al.*, 1993; Zobel *et al.*, 2001). Lower levels of CRF receptor binding sites and expression are also reported in the cerebral cortex of depressed suicide victims (Nemeroff *et al.*, 1988; Bissette *et al.*, 2003; Merali *et al.*, 2004). This may be due to a down-regulation of receptor signaling in response to excessive CRF release. Finally, there is a high incidence of depressive symptomatology in patients with Cushing's disease (Gibbons and McHugh, 1962; Carpenter and Bunney, 1971) and exacerbation of specific depressive disorders such as seasonal bipolar depression (De Bellis *et al.*, 1993;

Ghadirian *et al.*, 2005). Together, these data suggest that HPA axis dysfunction is a state, rather than a trait marker of depression which may be causally involved in the pathogenesis of this mood disorder. Further support for this hypothesis comes from work demonstrating the antidepressant properties of glucocorticoid synthesis inhibitors such as ketoconazole and metyrapone (Ghadirian *et al.*, 1995; Wolkowitz *et al.*, 1999). Ketoconazole alleviates depressive symptoms in hypercortisolemic but not in non-hypercortisolemic patients (Wolkowitz *et al.*, 1999).

Anxiety disorders and the HPA axis

Anxiety disorders too are linked with CRF-mediated dysregulation of the HPA axis. Evidence for the association between anxiety and HPA axis abnormalities is most established in post-traumatic stress (PTSD) and panic disorders. Elevated CRF levels in blood plasma (de Kloet *et al.*, 2008) and cerebrospinal fluid have been reported in PTSD patients (Bremner *et al.*, 1997; Baker *et al.*, 1999; Sautter *et al.*, 2003). Furthermore, PTSD patients also exhibit hypersuppression of cortisol in dexamethasone-suppression tests (de Kloet *et al.*, 2007) and hypersuppression of the ACTH response to cortisol (Yehuda *et al.*, 2006). Clinically, the use of pre-operative hydrocortisone treatment is protective against the development of PTSD (Schelling *et al.*, 2004). This association is also supported by preclinical work that demonstrates the reduction in the number of rats that display exaggerated anxiety-like behaviors post exposure to an olfactory stimulus stress when pretreated with corticosterone (Cohen *et al.*, 2006). Furthermore, glucocorticoids have also been shown to enhance extinction of fear responding in the rat and in human phobia patients (Soravia *et al.*, 2006; Yang *et al.*, 2006). In the case of generalized anxiety disorder, the involvement of the HPA axis is less clear. A number of

studies report no differences in CRF levels in cerebrospinal fluid of patients versus controls (Banki *et al.*, 1992; Jolkonen *et al.*, 1993; Fossey *et al.*, 1996). Another interesting piece of evidence implicating CRF in anxiety disorders is demonstrated by the association of a single nucleotide polymorphism in the CRF gene with behavioral inhibition (Smoller *et al.*, 2005); a heritable tempermental phenotype in children evidenced by fearful, avoidant or shy behaviour in novel situations. Behavioural inhibition is a risk factor for the development of panic disorder and social phobia (Isolan *et al.*, 2005).

Based on the evidence implicating dysfunction of the HPA axis in the aetiology of various psychiatric and stress-related disorders, CRF antagonists have been identified as a potential psychopharmaceuticals (Arborelius *et al.*, 1999; Grigoriadis, 2005; Van Den Eede *et al.*, 2005). Although, some of the psychotropic effects of such compounds may not be attributed to actions on the HPA axis (for review see Ising and Holsboer, 2007), these compounds reduce depressive symptoms (Zobel *et al.*, 2000). Improved understanding of the regulation of HPA axis function during basal and stressful conditions may hold the key to the development of new pharmacological approaches to the treatment of stress-related psychiatric disease.

Modulation of the HPA axis

A plethora of neurotransmitters and neuropeptides have been shown to modulate HPA axis activity and a comprehensive review is outside the scope of this review. Instead, we will briefly review regulation of the HPA axis by central serotonin and noradrenaline

since their regulation by imidazoline binding sites is well-documented and will be discussed in detail later.

Serotonergic modulation

Serotonergic (5-HT) neurons of the dorsal and medial raphe nucleus project to the PVN as well as the prefrontal cortex and other limbic and hypothalamic regions. The direct synaptic connections between 5-HT nerve terminals and the CRF-containing neurons of the PVN (Liposits *et al.*, 1987) permit both the inhibition and facilitation of HPA axis activity during basal and stressful conditions depending on the specific stressor (Saphier *et al.*, 1995), the receptor subtype activated (Welch *et al.*, 1993; Saphier and Welch, 1994; Saphier *et al.*, 1995) and route of administration (Welch and Saphier, 1994; Kageyama *et al.*, 1998; Samad *et al.*, 2006). Furthermore, chronic variation in circulating corticosterone alters the regulation of 5-HT neurones, increasing α_1 -adrenoceptor-mediated excitation and reducing 5-HT-mediated auto-inhibition at lower corticosterone levels (Judge *et al.*, 2004). This alteration has a major impact on control of 5-HT neuronal activity and hence HPA axis activity.

Noradrenergic modulation

Noradrenaline (NA) also plays a key role in regulating the HPA axis. Phasic, fear-associated stimuli produce robust increases in firing in locus coeruleus (LC) neurons and in turn, increase NA release in the brain (Valentino and Curtis, 1991). CRF fibers and receptors are found in the central arousal sympathetic systems including the LC-NA neurones and nucleus tractus solitarius (A2) in the brainstem (Swanson *et al.*, 1983). Electrical stimulation of the A1 or A2 region evokes excitatory responses in parvocellular

neurons in the PVN, whilst A6 evokes a more inhibitory response (Saphier and Feldman, 1989). Furthermore, electrical stimulation of the ventral noradrenergic ascending bundle, a fiber system primarily carrying catecholaminergic fibers arising from brainstem regions, increases hypophysial-portal plasma immunoreactive CRF levels (Plotsky, 1987). This suggests that the noradrenergic system plays an important stimulatory role in the modulation of HPA axis activity, and is further corroborated by work demonstrating a dose-dependent facilitation of CRF release by intracerebroventricular (i.c.v) administration of NA (Plotsky, 1987). This communication between NA and CRF fibers is bi-directional and it is believed that the CRF-containing neurons of the PVN and NA neurons of the brainstem comprise an autoregulatory negative feedback loop for the HPA axis (Silverman *et al.*, 1989; Calogero *et al.*, 1990).

A novel group of binding sites that modulate monoamine release have been identified and characterized, (for review see Eglen *et al.*, 1998). They have been classified as the imidazoline binding sites (IBS) (Ernsberger *et al.*, 1995). The effects of IBS on cardiovascular function have been well investigated since their discovery in 1984. However, evidence would also suggest a role in stress responsivity and psychiatric disease (Halaris and Piletz, 2003). The remainder of this review will deal with IBS, their importance in regulation of the stress response and their clinical relevance to psychiatric disorders.

IMIDAZOLINE BINDING SITES

The existence of imidazoline binding sites (IBS) was first proposed when clonidine and other compounds containing an imidazoline moiety were found to lower blood pressure

by acting at non-adrenoceptor sites in the brainstem (Bousquet *et al.*, 1984). These binding sites demonstrate a high affinity for imidazoline-class α_2 -adrenergic ligands but low affinity for known biogenic amines. Studies by several research groups indicate their presence in peripheral tissues and in both the central and peripheral nervous systems of various species (Campbell and Potter, 1994; Ernsberger and Haxhiu, 1997; Eglen *et al.*, 1998; MacInnes and Handley, 2005). Using radioligands such as [3 H]clonidine, [3 H]idazoxan, [3 H]harmane, [3 H]2-BFI and [3 H]BU224, three binding site subtypes have been identified: I₁, I₂ and I₃ which can be distinguished on the basis of pharmacology (Parini *et al.*, 1996; Mourtada *et al.*, 1997; Reis and Piletz, 1997; Eglen *et al.*, 1998), subcellular location (Tesson *et al.*, 1995; Ernsberger and Haxhiu, 1997; Zhang and Abdel-Rahman, 2006) and regional distribution (Ernsberger and Haxhiu, 1997; Lione *et al.*, 1998; Robinson *et al.*, 2002; MacInnes and Handley, 2005; Anderson *et al.*, 2006).

I₁ binding sites

I₁ IBS are characterised by their high affinity for 2-aminoimidazolines such as [3 H]clonidine, moderate affinity for imidazolines such as [3 H]idazoxan, and a low affinity for guanidines, for review see (Dardonville and Rozas, 2004). I₁ IBS have been identified in pre-synaptic neurons in the rostral ventrolateral medulla oblongata (RVLM) region (Ernsberger and Haxhiu, 1997) and are regionally distributed throughout the striatum, pallidum, dentate gyrus of the hippocampus, amygdala and substantia nigra (King *et al.*, 1995). Candidate proteins for this binding site have recently been cloned and include the imidazoline receptor antisera-selective (IRAS) protein and its mouse homologue, nischarin (Alahari *et al.*, 2000; Piletz *et al.*, 2000; Zhang and Abdel-Rahman, 2006; Sun

et al., 2007). IRAS/nischarin is a membrane-associated protein with a PX domain that binds to membrane phospholipids (Piletz *et al.*, 2000). An acidic region of similarity was found between IRAS-1 and the calcium-binding pore of ryanodine receptors (Piletz *et al.*, 2000). IRAS/nischarin is currently classified as a nexin or a scaffolding protein which participates in cell signalling (Ponting, 1996). Nischarin selectively inhibits Rac-driven signaling cascades that affect migration through p21-activated kinase (Alahari, 2003). In addition, nischarin can also regulate other elements of Rac1 signaling pathways (Reddig *et al.*, 2005). Studies investigating I₁ IBS signalling mechanisms have observed the activation of the phosphatidylcholine selective phospholipase C (PC-PLC) pathway (Ernsberger *et al.*, 1993; Renouard *et al.*, 1993; Separovic *et al.*, 1996; Separovic *et al.*, 1997; Takada *et al.*, 1997; Zhang *et al.*, 2001; Zhang and Abdel-Rahman, 2005) and increases in extracellular signal-regulated kinases and JNK by selective I₁ ligands in PC12 cells (Edwards *et al.*, 2001; Zhang *et al.*, 2001; Sano *et al.*, 2002; Sun *et al.*, 2007) as well as in vivo in the RVLM in the rat (Zhang and Abdel-Rahman, 2005). The activation of PC-PLC uniquely distinguishes I₁ IBS from neuronal α₂-adrenoceptors (Separovic *et al.*, 1997).

I₁ IBS is best known for its role as a regulator of blood pressure (Bousquet *et al.*, 1984; Chalmers and Pilowsky, 1991; Ernsberger *et al.*, 1995). High densities of I₁ IBS are localized in the RVLM, a region involved in cardiovascular activity. Evidence supporting a role for I₁ IBS in sympathoinhibition comes from work demonstrating that drugs devoid of affinity for α₂-adrenoceptors, but with affinity for I₁ IBS, lower blood pressure when injected into the RVLM (Bousquet *et al.*, 1984; Laubie *et al.*, 1985; Tibirica *et al.*, 1989).

Moreover, I₁ IBS antagonists block the cardiovascular effects of I₁ IBS agonists more effectively than pure α_2 -adrenoceptor antagonists (Wang *et al.*, 2007). I₁ ligands lower sympathetic tone by acting primarily on cardiovascular regulatory centres in the medulla. However, peripheral presynaptic inhibition of transmitter release from postganglionic sympathetic neurons contributes to the overall sympathoinhibition. The effects of imidazoline antihypertensives have been reviewed in detail by Szabo (2002) and Bousquet *et al.* (2003).

Alterations in I₁ IBS expression are evident in patients with depression which may suggest a role for these receptors in mediating some of the pathophysiological changes that occur in depression and other psychiatric disorders. We will re-visit this issue in greater detail later in the review.

I₂ binding sites

These binding sites are heterogenous in nature and have been further subdivided into I_{2A} and I_{2B} according to their sensitivity to amiloride (Olmos *et al.*, 1999). I₂ IBS are preferentially bound by imidazolines (Nutt *et al.*, 1995; Hudson *et al.*, 1997; Dardonville and Rozas, 2004) and show lower affinity for 2-aminoimidazolines (Eglen *et al.*, 1998). I₂ IBS are widely distributed in the central nervous system (CNS) and peripheral tissues. [³H]idazoxan, and more recently [³H]2-BFI and [³H]BU224, have proven to be important tools for elucidating the distribution of I₂ IBS. All three ligands possess high affinity for the I₂ IBS and the latter two possess a high degree of selectivity for I₂ IBS over α_2 -adrenoceptors and I₁ IBS (Dardonville and Rozas, 2004). Using receptor autoradiography, the highest densities of [³H]2-BFI binding (>90 fmol mg⁻¹ tissue) were observed in the arcuate nucleus, dorsal surface of the third ventricle, mammillary

peduncle, interpeduncular nucleus and area postrema (Lione *et al.*, 1998). Significant levels of binding are also found in the pineal gland, surface of fourth ventricle, ependyma, dorsal raphe and areas such as the hippocampus, frontal cortex and caudate-putamen. Similar regional binding was observed using [³H]BU224 and [³H]idazoxan (Robinson *et al.*, 2002). I₂ IBS are found largely on the outer membrane of mitochondria and possess a high degree of sequence homology with monoamine oxidases that reside there (Tesson *et al.*, 1995) This, along with evidence indicating that photolabelled I₂ IBS are immuno-precipitated with monoclonal MAO-A and MAO-B antibodies (Raddatz *et al.*, 1995) and a study showing that MAO enzymes cannot be separated from I₂ IBS during purification processes (Tesson *et al.*, 1995), suggests a strong relationship between the I₂ IBS and MAO.

[³H]Harmane and its analogs are particularly useful in elucidating the role of IBS on MAO as it acts as a specific and reversible inhibitor of the enzyme. Harmane is a potent inhibitor of MAO-A ($K_i = 55.5$ nM) but not MAO-B (Kim *et al.*, 1997; Herraiz and Chaparro, 2005). Harmine, 2-methylharminium, 2,9-dimethylharminium, and harmaline are also effective inhibitors of the purified MAO-A, with low K_i values of 5, 69, 15, and 48 nM, respectively (Kim *et al.*, 1997). However, residual [³H]harmane binding has been observed in both brain and kidney tissue from MAO-A knockout mice (Anderson *et al.*, 2006). This would suggest that although harmane is a potent inhibitor of MAO-A, it may also bind to a non-MAO site. There have also been other reports of a non-MAO associated I₂ IBS population. Immunoblotting experiments using a specific polyclonal antibody for IBS have found two peptides in human and rat brain with apparent molecular masses different from those reported for MAO-A (61 kDa) and MAO-B (55 kDa) (Escriba *et al.*, 1994). Furthermore, treatment of rats with the irreversible I₂ IBS

ligand BU99006 result in a decrease in [³H]2-BFI binding to brain membranes without any effect on MAO activity or on the binding of selective MAO-A or MAO-B radioligands (Paterson *et al.*, 2006). This would suggest that the site which binds BU99006 is not MAO-associated and further supports the existence of two distinct populations of I₂ sites, one which is associated with MAO and one which is not.

MAO inhibitors (MAOI) are sometimes prescribed to treat chronic depression in patients that do not respond to SSRIs and tricyclic antidepressants (TCAs). Alterations in MAO activity have been associated with mood disorders (Mayer *et al.*, 1976; Nash *et al.*, 2005; Yu *et al.*, 2005; Christiansen *et al.*, 2007; Giraldi *et al.*, 2007) and schizophrenia (Li and He, 2007). Interestingly, chronic MAOI treatment decreases expression of I₂ IBS in the brain (Olmos *et al.*, 1993). These findings implicate I₂ IBS and MAO enzyme activity with the pathogenesis of major depression and possibly other psychiatric disorders and we will present the evidence supporting this hypothesis later in the review.

I₃ binding site

The more recently identified I₃ insulinotropic sites are found in the pancreatic β cells (Morgan *et al.*, 1999). These sites are closely related to the adenosine 5' -triphosphate (ATP)-sensitive potassium channel, Kir6.2. Imidazolines can promote intracellular calcium mobilisation (Squires *et al.*, 2004) and directly initiate a mechanism of enhanced glucose-dependent insulin secretion (Mest *et al.*, 2001; Cooper *et al.*, 2003; Bleck *et al.*, 2004). A detailed review of I₃ imidazoline binding sites is outside the scope of the current article but for a comprehensive review see (Morgan and Chan, 2001).

Endogenous imidazoline binding site ligands

Clonidine displacing substance (CDS), the first crude isolation of an endogenous ligand for the imidazoline binding sites (Atlas, 1994), was associated with a number of biological functions including arterial pressure regulation (Bousquet *et al.*, 1986), smooth muscle contraction (Felsen *et al.*, 1987) and catecholamine release from adrenal chromaffin cells (Regunathan *et al.*, 1991). The components of CDS were later identified as tryptamine, agmatine and β carbolines: harmane and harmalan (Parker *et al.*, 2004). However, tryptamine's retention time, relative short-half life (Durden *et al.*, 1988) and tendency to convert into other active bioactive components suggested that it was not the bioactive component of classical CDS but rather a modulator of both α_2 -adrenoceptors and IBS (Parker *et al.*, 2004). Although agmatine is widely distributed throughout the body, binds to α_2 -adrenoceptors and IBS subtypes (Dardonville and Rozas, 2004) and possesses many qualities of a neurotransmitter (Reis and Regunathan, 2000), agmatine does not have the high potency or affinity at IBS nor diverse tissue distribution ascribed to the bioactive constituent of CDS (Parker *et al.*, 2000). Harmane and harmalan are the proposed bioactive constituents of CDS and the endogenous ligands for IBS (Parker *et al.*, 2004). Both harmane and harmalan have many of the functional characteristics associated with CDS and exhibit a high affinity for both the I₁ IBS and I₂ IBS. Harmane significantly reduces blood pressure by activating I₁ IBS (Musgrave and Badoer, 2000), modulates monoamine oxidases via the I₂ IBS (Albores *et al.*, 1990; Rommelspacher *et al.*, 1994; Kim *et al.*, 1997; Holt *et al.*, 2004) and increases insulin secretion in a glucose-dependent manner by activation of the I₃ IBS in pancreatic β -cells (Cooper *et al.*, 2003). Although, the physiological role of endogenous harmane has yet to be elucidated,

harmane and its β -carboline analogs are implicated in the modulation of body temperature (hyperthermia) (Adell *et al.*, 1996), the forebrain reward pathway (Ergene and Schoener, 1993) and fear-related behaviours (Kalin *et al.*, 1992; Talalaenko *et al.*, 2006). The latter topic is discussed in detail later.

Imidazoline-4-acetic acid ribotide (IAA-RP) has also been extracted from CDS (Prell *et al.*, 2004). IAA is a GABA_A receptor agonist that also exhibits a low efficacy, partial agonist effect at GABA_C receptors (Tunnicliff, 1998). Pulse–chase studies showed IAA could be conjugated with phosphoribosyl-pyrophosphate (while hydrolyzing ATP) to produce an IAA-ribotide (Fernandes *et al.*, 1960; Crowley, 1964). IAA-RP is thought to bind to I₁ IBS in adrenal tissue and promote the release of arachidonic acid from PC12 cells (Prell *et al.*, 2004). However, as a potential I₁ IBS agonist, one would expect IAA-RP administration into the RVLM to lower blood pressure, but in contrast, IAA-RP produces a rapid, transient increase in mean arterial pressure (Prell *et al.*, 2004). Similar to CDS and other I₃R agonists (Chan *et al.*, 1997; Eglen *et al.*, 1998), IAA-RP increased insulin secretion from islets (Prell *et al.*, 2004). Potentiation of glucose-induced insulin release is the best-characterized I₃R-mediated response (Chan *et al.*, 1997; Eglen *et al.*, 1998; Morgan *et al.*, 1999). I₃ IBS agonists also interact with K⁺ATP channels of the islets and these channels are found in high density in the RVLM (Golanov and Reis, 1999) and promote cell excitability (Chan *et al.*, 1997). These IAA-RP effects are characteristic of I₃R agonists. Interestingly, IAA-RP was more potent than the prototypical I₃ agonist /I₁ antagonist efavroxan EC₅₀ =30-50 nM (Prell *et al.*, 2004) versus 100 mM (Chan and Morgan, 1990; Chan *et al.*, 1997). Moreover, efavroxan induces hypertension when administered directly into the RVLM (Ernsberger, 1998). Thus, it is

speculated that IAA-RP may act at an I_3 IBS-like receptor located in the midbrain. Evidence also indicates that IAA-RP participates in trans-synaptic signalling in the brain. It is distributed throughout forebrain and limbic system regions including the amygdala, hypothalamus and hippocampus as well as in midbrain and brainstem nuclei (Friedrich *et al.*, 2007), exhibits depolarization-induced Ca^{2+} -dependent release from P_2 synaptosomal elements and possesses a relatively high affinity for membrane-bound IBS (Prell *et al.*, 2004). Furthermore IAA-RP is rapidly metabolised by phosphatases and ecto-5'-nucleotidases (Thomas and Prell, 1995). This would strongly suggest that not only is IAA-RP a proposed endogenous ligand for the I_3 IBS it may, in fact, act as a neurotransmitter.

The regulation of monoamines by imidazoline binding sites and their ligands

The modulation of noradrenergic neuron activity in the LC by IBS has been investigated. This is achieved by blocking the action of α_2 -adrenoceptors with N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ). Systemic administration of low doses of clonidine characteristically inhibits neuronal activity (Szabo, 2002). However, in EEDQ-treated rats, central administration of clonidine directly into the RVLM increases cell firing rate in the LC by $88 \pm 24\%$ versus basal firing (Ruiz-Ortega and Ugedo, 1997). Similarly, the characteristic inhibitory effect of low doses of clonidine on LC noradrenergic neurons was abolished and a paradoxical, dose-dependent increase in firing rate was observed at higher doses (Pineda *et al.*, 1993). In the same study, neuronal activation was also stimulated by cirazoline and rilmenidine; both I_1 IBS agonists. These results would suggest that IBS ligands can modulate the activity of LC noradrenergic neurones through the activation of I_1 IBS. However, the direct effect of these ligands on catecholaminergic

neurons in the LC is questionable. In a similar study, central administration of clonidine into the LC did not increase the firing rate of neurons in the EEDQ-pretreated anaesthetized rat (Ruiz-Ortega and Ugedo, 1997). Physiological recordings during this experiment showed there was no effect on blood pressure after clonidine administration in EEDQ-pretreated rats. Moreover, no functional IBS have been observed in midpontine slices prepared from the rat brain following application of clonidine, rilmenidine and moxonidine (Szabo and Urban, 1995). Thus, the effects of I₁ IBS ligands may be mediated by an indirect mechanism involving IBS located on RVLM neurons projecting to the LC.

Another study investigating the mechanisms underpinning the modulation of noradrenergic neuronal activity in the LC by clonidine has implicated an excitatory amino acid pathway, modulated by an inhibitory serotonergic mechanism. The stimulatory effect of clonidine on LC noradrenergic neurones in the rat is completely antagonized by pretreatment with kynurenic acid, an excitatory amino acid receptor antagonist (Ruiz-Ortega and Ugedo, 1997). In this study, clonidine-induced increases in LC noradrenergic neuronal activity are potentiated by both reserpine and p-chlorophenylalanine pretreatment (irreversible inhibitor of tryptophan hydroxylase, a rate-limiting enzyme in the biosynthesis of serotonin) but not with α -methyl-p-tyrosine (reversible inhibitor of tryptophan hydroxylase, a rate-limiting enzyme in the biosynthesis of catecholamines).

An important role for I₂ IBS in the modulation of brain monoamines has also been demonstrated. Both central and systemic administration of the selective I₂ ligand, 2-BFI,

increases the firing rate of LC cells in the anaesthetised rat (Ugedo *et al.*, 1998). This effect, however, is antagonized by chronic pretreatment with clorgyline, an irreversible monoamine oxidase inhibitor. In the same paper, rat midpontine brain slices containing the LC were bathed in 2-BFI, BU224 and idazoxan. Each agonist reversibly stimulated the LC cells, although when two agonists were administered simultaneously, cell firing did not increase in an additive fashion. When 2-BFI was applied to the bath, it reversed the inhibitory effects of ATP-sensitive K⁺ channel opener diazoxide. Furthermore, glibenclamide, an ATP-sensitive K⁺ channel blocker, partially blocked the effects of 2-BFI. This study indicates that the stimulation of LC cells by selective I₂ IBS ligands is not mediated by I₁ or I₂ IBS, despite the high density of I₂ IBS present in the LC (King *et al.*, 1995; MacKinnon *et al.*, 1995) but possibly via an IBS subtype yet to be identified. These data suggest that this IBS subtype is located extracellularly and modulates ATP-sensitive K⁺ channels.

IBS ligands also modulate the activity of dorsal raphe neurons in the rat. Harmane inhibits the firing of serotonergic dorsal raphe neurons (Ugedo *et al.*, 1999; Touiki *et al.*, 2005). It has been suggested that harmane achieves this by acting directly on specific neurotransmitter receptors (Muller *et al.*, 1981), although it possesses very low affinity for 5-HT_{2C} receptors and no affinity for 5-HT_{1A} receptors, dopamine D₂, or benzodiazepine receptors (Glennon *et al.*, 2000). The mechanism by which harmane inhibits serotonergic neurons still remains to be elucidated. Harmane and norharmane have also been implicated in the modulation of mesolimbic dopaminergic neurons (Ergene and Schoener, 1993). Here again, the precise pharmacological mechanisms have yet to be clarified.

Presynaptic IBS modulate the extracellular levels of monoamines by allosteric inhibition of MAO activity (Holt *et al.*, 2004). Although, IBS ligands were once thought to act only on peripheral noradrenergic cells (Gothert *et al.*, 1995; Molderings *et al.*, 1997), microdialysis studies show that systemic administration of selective IBS ligands increases extracellular levels of noradrenaline in the cerebral cortex (Meana *et al.*, 1997), the frontal cortex and hippocampus (Nutt *et al.*, 1995; Nutt *et al.*, 1997; Hudson *et al.*, 1999; Abu Ghazaleh *et al.*, 2007) and the hypothalamic PVN (Finn *et al.*, 2002). Similarly, I₂ IBS ligands increase extracellular levels of serotonin in the hippocampus and LC in the rat (Adell *et al.*, 1996; Ugedo *et al.*, 1999). I₂ IBS ligands also increase extracellular levels of dopamine in the striatum (Hudson *et al.*, 1999) and frontal cortex (Abu Ghazaleh *et al.*, 2007) of the rat. Table 1 and Figure 1 summarises the literature on the *in vivo* effects of IBS ligands on brain monoaminergic activity in rodents.

[Insert table 1 here] [Insert figure 1 here]

Regulation of the stress response by imidazoline binding sites

As discussed above, neurobiological systems mediating the stress response are subject to regulation by IBS. Moreover, there is direct evidence to suggest that IBS play a role in mediating and modulating neurochemical, neuroendocrine and behavioural responses to stress. The localisation of I₂ IBS in stress responsive brain regions (Lione *et al.*, 1998) and their modulation of monoamine levels via inhibition of MAO are of particular relevance to the stress response as monoamines play an essential role in HPA axis regulation. Consistent with a role for central NA in IBS-mediated modulation of HPA

axis function, we have demonstrated that systemic administration of the I₂ selective ligand BU224 significantly increased extracellular levels of NA in the rat PVN (Finn *et al.*, 2002). This elevation, as measured by microdialysis in awake, behaving rats, was observed at 20 and 40 minutes post injection (Figure 2). NA levels were also significantly increased by BU224 in rats with adjuvant-induced arthritis 20, 40 and 60 minutes post injection. The BU224-induced increase in extracellular levels of NA in this animal model of chronic inflammatory stress was significantly greater than that observed in non-stressed rats. In the same study, BU224 increased plasma corticosterone levels in both control and adjuvant-induced arthritis rats an increase which was positively correlated with the increase in extracellular NA. These data suggest that (a) I₂ IBS ligands stimulate HPA axis activity by increasing release of NA in the PVN and (b) the noradrenergic response to an I₂ IBS ligand is enhanced in the PVN of rats with adjuvant-induced arthritis. This indicates that I₂ IBS is an important pharmacological target as its activation facilitates NA release under basal and chronic stress conditions.

[Insert figure 2 here]

Selective I₂ IBS ligands have been shown to potentiate stress-induced neuroendocrine changes in other stress paradigms. We have shown previously that the plasma corticosterone response to acute psychological restraint stress in rats is enhanced by systemic administration of BU224 (Figure 3) and idazoxan compared with saline-treated restraint controls (Finn *et al.*, 2004). Thus, I₂ IBS ligands are capable of increasing corticosterone levels under basal conditions and during exposure to stress. In addition to the BU224-induced increase in NA in the PVN discussed above, further evidence indicating that I₂ IBS-mediated stimulation of HPA axis activity is centrally driven

comes from our work demonstrating that plasma ACTH levels were significantly increased by BU224 in both non-stressed rats and rats exposed to acute swim stress (Finn *et al.*, 2003). In addition, work with the endogenous IBS ligand harmaline has shown that expression of CRF mRNA is increased in the inferior olfactory complex of cats 8 hours following administration of this tremor-inducing β -carboline (Cummings *et al.*, 1994) although the relevance of this change to HPA axis activity is unclear. Interestingly, both preclinical and clinical trials show that plasma ACTH and glucocorticoids are significantly decreased by clonidine administration (Alexander and Irvine, 2000; Munoz-Hoyos *et al.*, 2000). This is continuous with the rationale that I₁ IBS activation may reduce monoaminergic activity in some brain regions and exert a consequent inhibitory effect on HPA axis activity. In contrast, acute administration of the I₂ IBS ligand idazoxan has been shown to attenuate the normal diurnal fall in plasma cortisol in humans (Glue *et al.*, 1992). Table 2 summarises the literature on modulation of stress hormones by imidazolines.

[Insert figure 3 here]

[Insert table 2 here]

Sympathetic activation is also an important part of the stress response as it initiates the physiological changes that enable the ‘fight or flight’ behavioural response. The I₁ IBS agonist moxonidine did not alter blood pressure in healthy human subjects at rest but decreased plasma noradrenaline levels (Wenzel *et al.*, 2004). Physical exercise using bicycle ergometry (50 watt increasing to 100, 150 and 200 watt every 2 min) significantly increased both blood pressure and plasma NA and this stress-induced increase in plasma NA, but not blood pressure, was attenuated with moxonidine pretreatment. However,

moxonidine pretreatment significantly reduces the stress-induced increase in both plasma NA and blood pressure exerted during mental stress testing (Wenzel *et al.*, 2004). Another study showed that moxonidine reduced blood pressure during driving simulation without a concomitant impairment of performance (Schmidt, 1992). These studies suggest that moxonidine decreases total sympathetic tone under basal and during physical exercise conditions as well as mental stress without limiting maximal exercise capacity. Similarly, rilmenidine, an I₁ IBS agonist reduces blood pressure in healthy and hypertensive resting subjects (Fauvel *et al.*, 1999; Esler *et al.*, 2004) and decreases peak exercise heart rate without modifying peak aerobic power (Teixeira de Castro *et al.*, 2006). Teixeira de Castro *et al.* (2006) have also shown that rilmenidine inhibits the stress-induced blood pressure increases exerted by mental stress testing (Teixeira de Castro *et al.*, 2006). Evidence with respect to the effects of rilmenidine on sympathetic nervous system activity during physical and mental stress, however, is equivocal and there are a number of studies which report that rilmendine does not alter responses in blood pressure and heart rate induced by physical (Fauvel *et al.*, 1999; Esler *et al.*, 2004) or mental (Esler *et al.*, 2004) stress. Moreover, studies in conscious rabbits have demonstrated that rilmenidine fails to inhibit sympathetic activation in response to noise stress psychological (Burke *et al.*, 1998; Head and Burke, 2004). Thus, modulation of sympathetic physiological responses by IBS ligands appear to be dependent on the type of ligand and stressor under investigation and further research in this area is warranted.

Preclinical evidence for potential therapeutic role of imidazoline binding sites in psychiatric disease

The effects of IBS ligands on behavioural responses to stress have also been investigated using animal models. The forced swim test (FST) is a predictive test of anti-depressant efficacy. In the FST, selective ligands for the I₂ IBS (BU224 and 2-BFI) significantly reduced the immobility time of rats compared with saline-treated controls (Nutt *et al.*, 1995; Finn *et al.*, 2002). These behavioural effects of BU224 were accompanied by modulation of both the HPA axis and brain monoamine tissue levels. This strongly implicates the I₂ IBS in mediating antidepressant-like behaviour. However, BU224 had no effect in the mouse FST (O'Neill *et al.*, 2001). Agmatine, which has moderate to high affinity for both the I₁ and I₂ IBS, reduced immobility time in the FST in both rats (1.25, 2.5 and 5 mg/kg s.c.) (Li *et al.*, 2003) and mice (20mg/kg s.c.) (Zomkowski *et al.*, 2002; Li *et al.*, 2003). The antidepressant-like effects of agmatine (10 mg/kg, i.p.) in the mouse FST can be blocked by pretreatment with efavoxan, idazoxan and antazoline (a ligand with high affinity for the I₂ IBS) (Zeidan *et al.*, 2007). Furthermore, subeffective dosing with 0.001 mg/kg, (i.p.) agmatine produces a synergistic antidepressant-like effect with clonidine, moxonidine, antazoline and MK-801 (a non-competitive NMDA receptor antagonist). Pretreatment of mice with yohimbine blocked the synergistic antidepressant-like effect of agmatine with clonidine. These data suggest that the anti-immobility effect of agmatine in the FST is dependent on its interaction with I₁ and I₂ IBS.

Agmatine also reduced immobility in the tail suspension test (TST) (40 and 80 mg/kg p.o.) (Zomkowski *et al.*, 2002; Li *et al.*, 2003) and elicited acute anxiolytic-like

behavioural changes in the rat and mouse in the elevated plus maze (EPM) following i.p. injection (10, 20, 40, 80, or 100 mg/kg, i.p), increasing time spent in the open arms, without accompanying changes in locomotor activity (Lavinsky *et al.*, 2003; Gong *et al.*, 2006). Moreover, harmane, the endogenous ligand for IBS also increases time spent in the open arms of the EPM (Aricioglu and Altunbas, 2003). However, the modulation of behaviours elicited by IBS ligands appears to be stressor specific. In contrast to the effects of IBS ligands in models that examine escape behaviour and learned helplessness, analogs of the endogenous ligand β -carboline potentiated fear-related behaviour and increased plasma levels of both ACTH and cortisol in infant rhesus monkeys when administered immediately after maternal separation (Kalin *et al.*, 1992). These differences may be due to the influence of other receptors activated by the particular compounds or possibly by modulatory effects stimulated by the psychological nature of the stressor. Differences in neuroendocrine and behavioural responses to physical versus psychological stressors have been previously reported (Martí and Armario, 1998)

Clinical evidence for involvement of imidazoline binding sites in pathogenesis of psychiatric disease

Alterations in binding site density

Additional evidence to suggest that IBS play a direct role in the pathology of stress-related disorders and psychiatric disease comes from work demonstrating alterations in binding site densities in psychiatric patients. For example, the density of I₂ IBS expression on platelet membranes is significantly reduced in platelets of depressed patients (Piletz *et al.*, 1994). Furthermore, I₂ IBS expression is altered in brain tissue. I₂ IBS are reduced by 40% in the frontal cortex of suicide victims (Sastre *et al.*, 1995).

Since the association between MAO and I₂ IBS is well established, studies investigating the correlation between MAO-B and suicidal behaviour are of particular relevance to the role of I₂-IBS in psychiatric disorders. Many of these studies, however, have reported negative findings, until a recent study showed that suicide victims expressed >30% increase in binding sites for lazabemide, an MAO-B inhibitor, in the frontal cortex versus matched controls (Ballesteros *et al.*, 2008). This study controlled for the influence of confounding variables such as age at death. Alterations in I₁ IBS densities have also been reported. Radioligand binding density (B_{max}) of I₁ IBS on platelet plasma membranes is elevated in depressed patients compared with healthy control subjects (Piletz *et al.*, 1994; Piletz *et al.*, 1996a).

Evidence from immuno-blotting experiments

Western blotting studies have correlated 33kDa and 85kDa bands found using imidazoline-binding protein antiserum with platelet I₁ IBS as detected by [¹²⁵I]p-iodoclonidine (Zhu *et al.*, 2003). Other bands have been identified and correlated with the I₁ IBS (Garcia-Sevilla *et al.*, 1999). Interestingly, immunoreactivity of these bands (35kDa and 45kDa) is upregulated in the platelets and brain membranes of depressed patients compared to matched controls (Garcia-Sevilla *et al.*, 1999). The 45kDa band was also upregulated in the brains of suicide victims compared to matched controls (Garcia-Sevilla *et al.*, 1996). In contrast, the immunoreactivity of both the 35kDa and 45kDa bands was decreased in hippocampal homogenates from depressed patients relative to matched controls (Piletz *et al.*, 2000). This brain region is renowned for morphological changes such as decreased neurogenesis and cellular remodeling, following stress

exposure (for a recent review Joca *et al.*, 2007). Surprisingly, no elevation of platelet I₁ IBS expression was evident in patients with generalized anxiety disorder (Piletz *et al.*, 1996a). Further correlations were also made between the immunoreactivity of the 45 kDa band and that of G $\alpha_{q/11}$, G α_{i2} and G β proteins (Garcia-Sevilla *et al.*, 1996). Such correlations suggest that the 45 kDa protein may couple to phosphoinositide pathway in platelets. This pathway has been postulated to function abnormally in mood disorders, for review see (Manji, 1992; Hudson *et al.*, 1993; Jope and Williams, 1994). More specifically, a 30% deficit in G protein-mediated [³H] PI hydrolysis (phosphoinositide signalling) has been reported in the prefrontal cortex of suicide victims with depression compared with matched controls (Pacheco *et al.*, 1996). These data highlight the functional implications of the abnormally high expression of I₁ IBS (45 kDa protein bands) and its importance in the pathogenesis of major depression and suicide.

Western blotting has also identified an 85 kDa band and correlated it with I₁ IBS (Ivanov *et al.*, 1998). The authors deduced that lower weight proteins identified using the same antiserum may be possible breakdown fragments of the 85 kDa band by omitting the mixture of protease inhibitors used to prepare the membranes. This 85 kDa band is up-regulated in response to administration of the IBS ligands moxonidine, idazoxan and agmatine (Ivanov *et al.*, 1998). Moreover, IRBP-immunoreactivity of the 85 kDa band is increased in the presence of NA and idazoxan whilst yohimbine, a non-imidazoline- α_2 antagonist, had no effect. This would suggest this protein is imidazoline specific and up-regulated only with a rise in NA levels and/or administration of IBS ligands.

IBS density and antidepressants

Importantly, all of the alterations observed are essentially reversible following antidepressant treatment (Piletz *et al.*, 1991; Garcia-Sevilla *et al.*, 1996; Piletz *et al.*, 1996b; Zhu *et al.*, 1997a; Zhu *et al.*, 1997b; Zhu *et al.*, 1999; Halaris and Piletz, 2001; Halaris *et al.*, 2002). Indeed, patients with bipolar depression that were treated with lithium showed normal IRBP-immunodensities (Garcia-Sevilla *et al.*, 1996; Garcia-Sevilla *et al.*, 1999). The down regulation of bands associated with I₁ IBS occurred only in depressed patients and not in healthy control subjects that received the antidepressant treatment (Piletz *et al.*, 2008). This would suggest that the down-regulation/normalization of platelet I₁ IBS is limited to treated depressed patients and is likely to be related to antidepressant-induced mood improvement. However, a platelet 33 kDa band is up-regulated during desipramine treatment of healthy subjects (Piletz *et al.*, 2008). This conflicts directly with findings in antidepressant-treated patients (Zhu *et al.*, 1999). This discrepancy supports observations by Ivanov *et al.* (1998) where they identified the 33 kDa band as a possible proteolytic breakdown product. Further investigation is required to elucidate the molecular nature of IBS and the intracellular mechanisms involved here.

CONCLUDING REMARKS

IBS-mediated modulation of central monoamines, HPA axis activity and stress-related behaviours strongly suggests a role for these novel targets in the pathophysiology and treatment of psychiatric disorders. Precisely how I₁ and I₂ IBS modulate the stress response is still relatively unclear, but with the availability of more selective synthetic ligands this task is becoming more achievable. Further work is also required to elucidate

the signaling mechanism and functional role of the I₂ IBS. This is essential to fully understand how this binding site is implicated in stress and might be manipulated for therapeutic effect. The physiological functions of the recently isolated endogenous ligands of IBS have also yet to be fully determined. The hope is that these findings will help us to develop more selective drugs with improved tolerability for patients with depression, anxiety and other stress-related psychiatric disorders. These binding sites offer a new and innovative target for the treatment of a myriad of stress-related disorders and psychiatric disease.

ACKNOWLEDGEMENTS

This work was supported by a grant from Science Foundation Ireland.

References

- Abu Ghazaleh H, Lalias MD, Husbands SM, Nutt DJ and Hudson AL 2007. The effect of 1-(4,5-dihydro-1H-imidazol-2-yl) isoquinoline on monoamine release and turnover in the rat frontal cortex. *Neurosci Lett*, **422**: 109-113.
- Adell A, Biggs TA and Myers RD 1996. Action of harman (1-methyl-beta-carboline) on the brain: body temperature and in vivo efflux of 5-HT from hippocampus of the rat. *Neuropharmacology*, **35**: 1101-1107.
- Alahari SK 2003. Nischarin inhibits Rac induced migration and invasion of epithelial cells by affecting signaling cascades involving PAK. *Exp Cell Res*, **288**: 415-424.
- Alahari SK, Lee JW and Juliano RL 2000. Nischarin, a novel protein that interacts with the integrin alpha5 subunit and inhibits cell migration. *J Cell Biol*, **151**: 1141-1154.
- Albores R, Neafsey EJ, Drucker G, Fields JZ and Collins MA 1990. Mitochondrial respiratory inhibition by N-methylated beta-carboline derivatives structurally resembling N-methyl-4-phenylpyridine. *Proc Natl Acad Sci U S A*, **87**: 9368-9372.
- Alexander SL and Irvine CH 2000. The effect of the alpha-2-adrenergic agonist, clonidine, on secretion patterns and rates of adrenocorticotrophic hormone and its secretagogues in the horse. *J Neuroendocrinol*, **12**: 874-880.
- Anderson NJ, Seif I, Nutt DJ, Hudson AL and Robinson ES 2006. Autoradiographical distribution of imidazoline binding sites in monoamine oxidase A deficient mice. *J Neurochem*, **96**: 1551-1559.
- Arborelius L, Owens MJ, Plotsky PM and Nemeroff CB 1999. The role of corticotropin-releasing factor in depression and anxiety disorders. *J Endocrinol*, **160**: 1-12.

- Aricioglu F and Altunbas H 2003. Harmane induces anxiolysis and antidepressant-like effects in rats. *Ann N Y Acad Sci*, **1009**: 196-201.
- Atlas D 1994. Identifying clonidine-displacing substance. *Science*, **266**: 462-464.
- Baker DG, West SA, Nicholson WE, Ekhator NN, Kasckow JW, Hill KK, Bruce AB, Orth DN and Geraciotti TD, Jr. 1999. Serial CSF corticotropin-releasing hormone levels and adrenocortical activity in combat veterans with posttraumatic stress disorder. *Am J Psychiatry*, **156**: 585-588.
- Ballesteros J, Maeztu AI, Callado LF, Meana JJ and Gutierrez M 2008. Specific binding of [(3)H]Ro 19-6327 (lazabemide) to monoamine oxidase B is increased in frontal cortex of suicide victims after controlling for age at death. *Eur Neuropsychopharmacol*, **18**: 55-61.
- Banki CM, Karmacs L, Bissette G and Nemeroff CB 1992. CSF corticotropin-releasing hormone and somatostatin in major depression: response to antidepressant treatment and relapse. *Eur Neuropsychopharmacol*, **2**: 107-113.
- Bissette G, Klimek V, Pan J, Stockmeier C and Ordway G 2003. Elevated concentrations of CRF in the locus coeruleus of depressed subjects. *Neuropsychopharmacology*, **28**: 1328-1335.
- Bleck C, Wienbergen A and Rustenbeck I 2004. Glucose dependence of imidazoline-induced insulin secretion: different characteristics of two ATP-Sensitive K⁺ channel-blocking compounds. *Diabetes*, **53 Suppl 3**: S135-139.
- Bornstein SR, Engeland WC, Ehrhart-Bornstein M and Herman JP 2008. Dissociation of ACTH and glucocorticoids. *Trends Endocrinol Metab*.

- Bousquet P, Greney H, Bruban V, Schann S, Ehrhardt JD, Monassier L and Feldman J 2003. I(1) imidazoline receptors involved in cardiovascular regulation: where are we and where are we going? *Ann N Y Acad Sci*, **1009**: 228-233.
- Bousquet P, Feldman J and Atlas D 1986. An endogenous, non-catecholamine clonidine antagonist increases mean arterial blood pressure. *Eur J Pharmacol*, **124**: 167-170.
- Bousquet P, Feldman J and Schwartz J 1984. Central cardiovascular effects of alpha adrenergic drugs: differences between catecholamines and imidazolines. *J Pharmacol Exp Ther*, **230**: 232-236.
- Bremner JD, Licinio J, Darnell A, Krystal JH, Owens MJ, Southwick SM, Nemeroff CB and Charney DS 1997. Elevated CSF corticotropin-releasing factor concentrations in posttraumatic stress disorder. *Am J Psychiatry*, **154**: 624-629.
- Brown CM, MacKinnon AC, Redfern WS, Williams A, Linton C, Stewart M, Clague RU, Clark R and Spedding M 1995. RS-45041-190: a selective, high-affinity ligand for I2 imidazoline receptors. *Br J Pharmacol*, **116**: 1737-1744.
- Burke SL, Malpas SC and Head GA 1998. Effect of rilmenidine on the cardiovascular responses to stress in the conscious rabbit. *J Auton Nerv Syst*, **72**: 177-186.
- Calogero AE, Bagdy G, Szemeredi K, Tartaglia ME, Gold PW and Chrousos GP 1990. Mechanisms of serotonin receptor agonist-induced activation of the hypothalamic-pituitary-adrenal axis in the rat. *Endocrinology*, **126**: 1888-1894.
- Campbell WR and Potter DE 1994. Potential role of imidazoline (I1) receptors in modulating aqueous humor dynamics. *J Ocul Pharmacol*, **10**: 393-402.
- Carpenter WT, Jr. and Bunney WE, Jr. 1971. Behavioral effects of cortisol in man. *Semin Psychiatry*, **3**: 421-434.

- Chalmers J and Pilowsky P 1991. Brainstem and bulbospinal neurotransmitter systems in the control of blood pressure. *J Hypertens*, **9**: 675-694.
- Chan SL and Morgan NG 1990. Stimulation of insulin secretion by efaxoroxan may involve interaction with potassium channels. *Eur J Pharmacol*, **176**: 97-101.
- Chan SL, Pallett AL and Morgan NG 1997. Clotrimazole and efaxoroxan stimulate insulin secretion by different mechanisms in rat pancreatic islets. *Naunyn Schmiedebergs Arch Pharmacol*, **356**: 763-768.
- Christiansen L, Tan Q, Iachina M, Bathum L, Kruse TA, McGue M and Christensen K 2007. Candidate gene polymorphisms in the serotonergic pathway: influence on depression symptomatology in an elderly population. *Biol Psychiatry*, **61**: 223-230.
- Chrousos GP and Gold PW 1992. The concepts of stress and stress system disorders. Overview of physical and behavioral homeostasis. *Jama*, **267**: 1244-1252.
- Cohen H, Zohar J, Gidron Y, Matar MA, Belkind D, Loewenthal U, Kozlovsky N and Kaplan Z 2006. Blunted HPA axis response to stress influences susceptibility to posttraumatic stress response in rats. *Biol Psychiatry*, **59**: 1208-1218.
- Connor TJ, Kelly JP and Leonard BE 1997. Forced swim test-induced neurochemical endocrine, and immune changes in the rat. *Pharmacol Biochem Behav*, **58**: 961-967.
- Cooper EJ, Hudson AL, Parker CA and Morgan NG 2003. Effects of the beta-carbolines, harmine and pinoline, on insulin secretion from isolated human islets of Langerhans. *Eur J Pharmacol*, **482**: 189-196.
- Crowley GM 1964. The Enzymatic Synthesis of 5'-Phosphoribosylimidazoleacetic Acid. *J Biol Chem*, **239**: 2593-2601.

Cummings S, Hinds D and Young WS, 3rd 1994. Corticotropin-releasing factor mRNA increases in the inferior olfactory complex during harmaline-induced tremor. *Brain Res*, **660**: 199-208.

Dardonville C and Rozas I 2004. Imidazoline binding sites and their ligands: an overview of the different chemical structures. *Med Res Rev*, **24**: 639-661.

Day R, Nielsen JA, Korten A, Ernberg G, Dube KC, Gebhart J, Jablensky A, Leon C, Marsella A, Olatawura M and et al. 1987. Stressful life events preceding the acute onset of schizophrenia: a cross-national study from the World Health Organization. *Cult Med Psychiatry*, **11**: 123-205.

De Bellis MD, Gold PW, Geraciotti TD, Jr., Listwak SJ and Kling MA 1993. Association of fluoxetine treatment with reductions in CSF concentrations of corticotropin-releasing hormone and arginine vasopressin in patients with major depression. *Am J Psychiatry*, **150**: 656-657.

de Kloet CS, Vermetten E, Geuze E, Lentjes EG, Heijnen CJ, Stalla GK and Westenberg HG 2008. Elevated plasma corticotrophin-releasing hormone levels in veterans with posttraumatic stress disorder. *Prog Brain Res*, **167**: 287-291.

de Kloet CS, Vermetten E, Heijnen CJ, Geuze E, Lentjes EG and Westenberg HG 2007. Enhanced cortisol suppression in response to dexamethasone administration in traumatized veterans with and without posttraumatic stress disorder. *Psychoneuroendocrinology*, **32**: 215-226.

Diaz A, Mayet S and Dickenson AH 1997. BU-224 produces spinal antinociception as an agonist at imidazoline I₂ receptors. *Eur J Pharmacol*, **20**: 9-15.

Dubrovsky B 2000. The specificity of stress responses to different nocuous stimuli: neurosteroids and depression. *Brain Res Bull*, **51**: 443-455.

Durden DA, Nguyen TV and Boulton AA 1988. Kinetics of intraventricularly injected trace amines and their deuterated isotopomers. *Neurochem Res*, **13**: 943-950.

Edwards L, Fishman D, Horowitz P, Bourbon N, Kester M and Ernsberger P 2001. The I1-imidazoline receptor in PC12 pheochromocytoma cells activates protein kinases C, extracellular signal-regulated kinase (ERK) and c-jun N-terminal kinase (JNK). *J Neurochem*, **79**: 931-940.

Eglen RM, Hudson AL, Kendall DA, Nutt DJ, Morgan NG, Wilson VG and Dillon MP 1998. 'Seeing through a glass darkly': casting light on imidazoline T sites. *Trends Pharmacol Sci*, **19**: 381-390.

Ergene E and Schoener EP 1993. Effects of harmane (1-methyl-beta-carboline) on neurons in the nucleus accumbens of the rat. *Pharmacol Biochem Behav*, **44**: 951-957.

Ernsberger P 1998. Arachidonic acid release from PC12 pheochromocytoma cells is regulated by I1-imidazoline receptors. *J Auton Nerv Syst*, **72**: 147-154.

Ernsberger P, Damon TH, Graff LM, Schafer SG and Christen MO 1993. Moxonidine, a centrally acting antihypertensive agent, is a selective ligand for I1-imidazoline sites. *J Pharmacol Exp Ther*, **264**: 172-182.

Ernsberger P, Graves ME, Graff LM, Zaki N, Nguyen P, Collins LA, Westbrooks KL and Johnson GG 1995. I1-imidazoline receptors. Definition, characterization, distribution, and transmembrane signaling. *Ann NY Acad Sci*, **763**: 22-42.

Ernsberger P and Haxhiu MA 1997. The I1-imidazoline-binding site is a functional receptor mediating vasodepression via the ventral medulla. *Am J Physiol*, **273**: R1572-1579.

- Escriba PV, Sastre M, Wang H, Regunathan S, Reis DJ and Garcia-Sevilla JA 1994. Immunodetection of putative imidazoline receptor proteins in the human and rat brain and other tissues. *Neurosci Lett*, **178**: 81-84.
- Esler M, Lux A, Jennings G, Hastings J, Socratous F and Lambert G 2004. [Rilmenidine sympatholytic activity preserves mental and orthostatic sympathetic response and epinephrine secretion]. *Arch Mal Coeur Vaiss*, **97**: 786-792.
- Fauvel JP, Najem R, Ryon B, Ducher M and Laville M 1999. Effects of rilmenidine on stress-induced peak blood pressure and renal function. *J Cardiovasc Pharmacol*, **34**: 41-45.
- Felsen D, Ernsberger P, Meeley MP and Reis DJ 1987. Clonidine displacing substance is biologically active on smooth muscle. *Eur J Pharmacol*, **142**: 453-455.
- Fernandes JF, Castellani O and Plesé M 1960. Biosynthesis of histamine ribotide and imidazoleacetate ribotide. *Biochem Biophys Res Commun*, **3**: 679-684.
- Finlay-Jones R and Brown GW 1981. types of stressful life event and the onset of anxiety and depressive disorders. *Psychol Med*, **11**: 803-815.
- Finn DP, Hudson AL, Kinoshita H, Coventry TL, Jessop DS, Nutt DJ and Harbuz MS 2004. Imidazoline2 (I2) receptor- and alpha2-adrenoceptor-mediated modulation of hypothalamic-pituitary-adrenal axis activity in control and acute restraint stressed rats. *J Psychopharmacol*, **18**: 47-53.
- Finn DP, Lalias MD, Harbuz MS, Jessop DS, Hudson AL and Nutt DJ 2002. Imidazoline(2) (I(2)) binding site- and alpha(2)-adrenoceptor-mediated modulation of central noradrenergic and HPA axis function in control rats and chronically stressed rats with adjuvant-induced arthritis. *Neuropharmacology*, **42**: 958-965.

Finn DP, Marti O, Harbuz MS, Valles A, Belda X, Marquez C, Jessop DS, Lalies MD, Armario A, Nutt DJ and Hudson AL 2003. Behavioral, neuroendocrine and neurochemical effects of the imidazoline I2 receptor selective ligand BU224 in naive rats and rats exposed to the stress of the forced swim test. *Psychopharmacology (Berl)*, **167**: 195-202.

Fossey MD, Lydiard RB, Ballenger JC, Laraia MT, Bissette G and Nemeroff CB 1996. Cerebrospinal fluid corticotropin-releasing factor concentrations in patients with anxiety disorders and normal comparison subjects. *Biol Psychiatry*, **39**: 703-707.

Friedrich VL, Jr., Martinelli GP, Prell GD and Holstein GR 2007. Distribution and cellular localization of imidazoleacetic acid-ribotide, an endogenous ligand at imidazol(in)e and adrenergic receptors, in rat brain. *J Chem Neuroanat*, **33**: 53-64.

Garcia-Sevilla JA, Escriba PV and Guimon J 1999. Imidazoline receptors and human brain disorders. *Ann N Y Acad Sci*, **881**: 392-409.

Garcia-Sevilla JA, Escriba PV, Sastre M, Walzer C, Busquets X, Jaquet G, Reis DJ and Guimon J 1996. Immunodetection and quantitation of imidazoline receptor proteins in platelets of patients with major depression and in brains of suicide victims. *Arch Gen Psychiatry*, **53**: 803-810.

Gargalidis-Moudanos C and Parini A 1995. Selectivity of rilmenidine for I1-imidazoline-binding sites in rabbit proximal tubule cells. *J Cardiovasc Pharmacol*, **26 Suppl 2**: S59-62.

Ghadirian AM, Engelmann F, Dhar V, Filipini D, Keller R, Chouinard G and Murphy BE 1995. The psychotropic effects of inhibitors of steroid biosynthesis in depressed patients refractory to treatment. *Biol Psychiatry*, **37**: 369-375.

- Ghadirian AM, Marcovitz S and Pearson Murphy BE 2005. A case of seasonal bipolar disorder exacerbated by Cushing's disease. *Compr Psychiatry*, **46**: 155-158.
- Gibbons JL and McHugh Pr 1962. Plasma cortisol in depressive illness. *J Psychiatr Res*, **1**: 162-171.
- Giraldi T, De Vanna M, Malagoli M, Tuveri G, Sutto K, Schillani G and Grassi L 2007. Mental adaptation to cancer: depression and blood platelet monoamine oxidase activity in breast cancer patients. *Anticancer Res*, **27**: 1715-1719.
- Glennon RA, Dukat M, Grella B, Hong S, Costantino L, Teitler M, Smith C, Egan C, Davis K and Mattson MV 2000. Binding of beta-carbolines and related agents at serotonin (5-HT(2) and 5-HT(1A)), dopamine (D(2)) and benzodiazepine receptors. *Drug Alcohol Depend*, **60**: 121-132.
- Glue P, Wilson S, Campling GM, Knightly M, Franklin M, Cowen PJ and Nutt DJ 1992. Alpha-2-adrenoceptor control of cortisol and ACTH in normal volunteers: preliminary open trial of the effects of acute and chronic idazoxan. *Psychoneuroendocrinology*, **17**: 261-266.
- Golanov EV and Reis DJ 1999. A role for KATP+-channels in mediating the elevations of cerebral blood flow and arterial pressure by hypoxic stimulation of oxygen-sensitive neurons of rostral ventrolateral medulla. *Brain Res*, **827**: 210-214.
- Gollan JK, Lee R and Coccaro EF 2005. Developmental psychopathology and neurobiology of aggression. *Dev Psychopathol*, **17**: 1151-1171.
- Gong ZH, Li YF, Zhao N, Yang HJ, Su RB, Luo ZP and Li J 2006. Anxiolytic effect of agmatine in rats and mice. *Eur J Pharmacol*, **550**: 112-116.
- Gothert M, Molderings GJ, Fink K and Schlicker E 1995. Alpha 2-adrenoceptor-independent inhibition by imidazolines and guanidines of noradrenaline release

- from peripheral, but not central noradrenergic neurons. *Ann N Y Acad Sci*, **763**: 405-419.
- Greden JF, Gardner R, King D, Grunhaus L, Carroll BJ and Kronfol Z 1983. Dexamethasone suppression tests in antidepressant treatment of melancholia. The process of normalization and test-retest reproducibility. *Arch Gen Psychiatry*, **40**: 493-500.
- Grigoriadis DE 2005. The corticotropin-releasing factor receptor: a novel target for the treatment of depression and anxiety-related disorders. *Expert Opin Ther Targets*, **9**: 651-684.
- Gruen R and Baron M 1984. Stressful life events and schizophrenia. Relation to illness onset and family history. *Neuropsychobiology*, **12**: 206-208.
- Halaris A and Piletz JE 2001. Imidazoline receptors: possible involvement in the pathophysiology and treatment of depression. *Hum Psychopharmacol*, **16**: 65-69.
- Halaris A and Piletz JE 2003. Relevance of imidazoline receptors and agmatine to psychiatry: a decade of progress. *Ann N Y Acad Sci*, **1009**: 1-20.
- Halaris A, Zhu H, Ali J, Nasrallah A, Lindsay De Vane C and Piletz JE 2002. Down-regulation of platelet imidazoline-1-binding sites after bupropion treatment. *Int J Neuropsychopharmacol*, **5**: 37-46.
- Head GA and Burke SL 2004. Sympathetic responses to stress and rilmenidine in 2K1C rabbits: evidence of enhanced nonvascular effector mechanism. *Hypertension*, **43**: 636-642.
- Heim C, Plotsky PM and Nemeroff CB 2004. Importance of studying the contributions of early adverse experience to neurobiological findings in depression. *Neuropsychopharmacology*, **29**: 641-648.

- Herraiz T and Chaparro C 2005. Human monoamine oxidase is inhibited by tobacco smoke: beta-carboline alkaloids act as potent and reversible inhibitors. *Biochem Biophys Res Commun*, **326**: 378-386.
- Holsboer F 2000. The corticosteroid receptor hypothesis of depression. *Neuropsychopharmacology*, **23**: 477-501.
- Holt A, Wieland B and Baker GB 2004. Allosteric modulation of semicarbazide-sensitive amine oxidase activities in vitro by imidazoline receptor ligands. *Br J Pharmacol*, **143**: 495-507.
- Hudson AL, Chapleo CB, Lewis JW, Husbands S, Grivas K, Mallard NJ and Nutt DJ 1997. Identification of ligands selective for central I2-imidazoline binding sites. *Neurochem Int*, **30**: 47-53.
- Hudson AL, Gough R, Tyacke R, Lione L, Lalies M, Lewis J, Husbands S, Knight P, Murray F, Hutson P and Nutt DJ 1999. Novel selective compounds for the investigation of imidazoline receptors. *Ann N Y Acad Sci*, **881**: 81-91.
- Hudson CJ, Young LT, Li PP and Warsh JJ 1993. CNS signal transduction in the pathophysiology and pharmacotherapy of affective disorders and schizophrenia. *Synapse*, **13**: 278-293.
- Ising M and Holsboer F 2007. CRH-sub-1 receptor antagonists for the treatment of depression and anxiety. *Exp Clin Psychopharmacol*, **15**: 519-528.
- Isolan LR, Zeni CP, Mezzomo K, Blaya C, Kipper L, Heldt E and Manfro GG 2005. Behavioral inhibition and history of childhood anxiety disorders in Brazilian adult patients with panic disorder and social anxiety disorder. *Rev Bras Psiquiatr*, **27**: 97-100.

- Ivanov TR, Zhu H, Regunathan S, Reis DJ, Dontenwill M, Vontron C, Bousquet P and Piletz JE 1998. Co-detection by two imidazoline receptor protein antisera of a novel 85 kilodalton protein. *Biochem Pharmacol*, **55**: 649-655.
- Joca SR, Ferreira FR and Guimaraes FS 2007. Modulation of stress consequences by hippocampal monoaminergic, glutamatergic and nitrergic neurotransmitter systems. *Stress*, **10**: 227-249.
- Jolkonen J, Lepola U, Bissette G, Nemeroff C and Riekkinen P 1993. CSF corticotropin-releasing factor is not affected in panic disorder. *Biol Psychiatry*, **33**: 136-138.
- Jope RS and Williams MB 1994. Lithium and brain signal transduction systems. *Biochem Pharmacol*, **47**: 429-441.
- Judge SJ, Ingram CD and Gartside SE 2004. Moderate differences in circulating corticosterone alter receptor-mediated regulation of 5-hydroxytryptamine neuronal activity. *J Psychopharmacol*, **18**: 475-483.
- Kageyama K, Tozawa F, Horiba N, Watanobe H and Suda T 1998. Serotonin stimulates corticotropin-releasing factor gene expression in the hypothalamic paraventricular nucleus of conscious rats. *Neurosci Lett*, **243**: 17-20.
- Kalin NH, Shelton SE and Turner JG 1992. Effects of beta-carboline on fear-related behavioral and neurohormonal responses in infant rhesus monkeys. *Biol Psychiatry*, **31**: 1008-1019.
- Kendler KS and Karkowski-Shuman L 1997. Stressful life events and genetic liability to major depression: genetic control of exposure to the environment? *Psychol Med*, **27**: 539-547.

- Kendler KS, Karkowski LM and Prescott CA 1999. Causal relationship between stressful life events and the onset of major depression. *Am J Psychiatry*, **156**: 837-841.
- Kim H, Sablin SO and Ramsay RR 1997. Inhibition of monoamine oxidase A by beta-carboline derivatives. *Arch Biochem Biophys*, **337**: 137-142.
- King PR, Gundlach AL and Louis WJ 1995. Quantitative autoradiographic localization in rat brain of alpha 2-adrenergic and non-adrenergic I-receptor binding sites labelled by [3H]rilmenidine. *Brain Res*, **675**: 264-278.
- Krishnan KR, Doraiswamy PM, Lurie SN, Figiel GS, Husain MM, Boyko OB, Ellinwood EH, Jr. and Nemeroff CB 1991. Pituitary size in depression. *J Clin Endocrinol Metab*, **72**: 256-259.
- Lahniers CE and White K 1976. Changes in environmental life events and their relationship to psychiatric hospital admissions. *J Nerv Ment Dis*, **163**: 154-158.
- Laubie M, Poignant JC, Scuvee-Moreau J, Dabire H, Dresse A and Schmitt H 1985. Pharmacological properties of (N-dicyclopropylmethyl) amino-2-oxazoline (S 3341), an alpha-2 adrenoceptor agonist. *J Pharmacol*, **16**: 259-278.
- Lavinsky D, Arteni NS and Netto CA 2003. Agmatine induces anxiolysis in the elevated plus maze task in adult rats. *Behav Brain Res*, **141**: 19-24.
- Li D and He L 2007. Meta-study on association between the monoamine oxidase A gene (MAOA) and schizophrenia. *Am J Med Genet B Neuropsychiatr Genet*.
- Li YF, Gong ZH, Cao JB, Wang HL, Luo ZP and Li J 2003. Antidepressant-like effect of agmatine and its possible mechanism. *Eur J Pharmacol*, **469**: 81-88.
- Lione LA, Nutt DJ and Hudson AL 1998. Characterisation and localisation of [3H]2-(2-benzofuranyl)-2-imidazoline binding in rat brain: a selective ligand for imidazoline I2 receptors. *Eur J Pharmacol*, **353**: 123-135.

- Liposits Z, Uht RM, Harrison RW, Gibbs FP, Paull WK and Bohn MC 1987. Ultrastructural localization of glucocorticoid receptor (GR) in hypothalamic paraventricular neurons synthesizing corticotropin releasing factor (CRF). *Histochemistry*, **87**: 407-412.
- MacInnes N and Handley SL 2005. Autoradiographic localisation of [³H]2-BFI imidazoline I₂ binding sites in mouse brain. *Eur J Pharmacol*, **516**: 139-144.
- MacKinnon AC, Redfern WS and Brown CM 1995. [³H]-RS-45041-190: a selective high-affinity radioligand for I₂ imidazoline receptors. *Br J Pharmacol*, **116**: 1729-1736.
- Manji HK 1992. G proteins: implications for psychiatry. *Am J Psychiatry*, **149**: 746-760.
- Marti O and Armario A 1998. Anterior pituitary response to stress: time-related changes and adaptation. *Int J Dev Neurosci*, **16**: 241-260.
- Mayer KH, Stamler J, Dyer A, Freinkel N, Stamler R, Berkson DM and Farber B 1976. Epidemiologic findings on the relationship of time of day and time since last meal to glucose tolerance. *Diabetes*, **25**: 936-943.
- McEwen BS 2007. Physiology and neurobiology of stress and adaptation: central role of the brain. *Physiol Rev*, **87**: 873-904.
- Meana JJ, Herrera-Marschitz M, Goiny M and Silveira R 1997. Modulation of catecholamine release by alpha 2-adrenoceptors and I₁-imidazoline receptors in rat brain. *Brain Res*, **744**: 216-226.
- Merali Z, Du L, Hrdina P, Palkovits M, Faludi G, Poulter MO and Anisman H 2004. Dysregulation in the suicide brain: mRNA expression of corticotropin-releasing hormone receptors and GABA(A) receptor subunits in frontal cortical brain region. *J Neurosci*, **24**: 1478-1485.

- Mest HJ, Raap A, Schloos J, Treinies I, Paal M, Giese U and Koivisto V 2001. Glucose-induced insulin secretion is potentiated by a new imidazoline compound. *Naunyn Schmiedebergs Arch Pharmacol*, **364**: 47-52.
- Michelson D, Galliven E, Hill L, Demitack M, Chrousos G and Gold P 1997. Chronic imipramine is associated with diminished hypothalamic-pituitary-adrenal axis responsivity in healthy humans. *J Clin Endocrinol Metab*, **82**: 2601-2606.
- Modell S, Yassouridis A, Huber J and Holsboer F 1997. Corticosteroid receptor function is decreased in depressed patients. *Neuroendocrinology*, **65**: 216-222.
- Molderings GJ, Likungu J, Jakschik J and Gothert M 1997. Presynaptic imidazoline receptors and non-adrenoceptor [³H]-idazoxan binding sites in human cardiovascular tissues. *Br J Pharmacol*, **122**: 43-50.
- Morgan NG and Chan SL 2001. Imidazoline binding sites in the endocrine pancreas: can they fulfil their potential as targets for the development of new insulin secretagogues? *Curr Pharm Des*, **7**: 1413-1431.
- Morgan NG, Chan SL, Mourtada M, Monks LK and Ramsden CA 1999. Imidazolines and pancreatic hormone secretion. *Ann N Y Acad Sci*, **881**: 217-228.
- Mourtada M, Smith SA and Morgan NG 1997. Insulin secretagogues with an imidazoline structure inhibit arginine-induced secretion from isolated glucagon secretion from isolated rat islets of Langerhans. *Biochem Biophys Res Commun*, **236**: 162-166.
- Muller WE, Fehske KJ, Borbe HO, Wollert U, Nanz C and Rommelspacher H 1981. On the neuropharmacology of harmaine and other beta-carbolines. *Pharmacol Biochem Behav*, **14**: 693-699.

- Munoz-Hoyos A, Fernandez-Garcia JM, Molina-Carballo A, Macias M, Escames G, Ruiz-Cosano C and Acuna-Castroviejo D 2000. Effect of clonidine on plasma ACTH, cortisol and melatonin in children. *J Pineal Res*, **29**: 48-53.
- Musgrave IF and Badoer E 2000. Harmane produces hypotension following microinjection into the RVLM: possible role of I(1)-imidazoline receptors. *Br J Pharmacol*, **129**: 1057-1059.
- Nash MW, Sugden K, Huezo-Diaz P, Williamson R, Sterne A, Purcell S, Sham PC and Craig IW 2005. Association analysis of monoamine genes with measures of depression and anxiety in a selected community sample of siblings. *Am J Med Genet B Neuropsychiatr Genet*, **135**: 33-37.
- Nemeroff CB, Owens MJ, Bissette G, Andorn AC and Stanley M 1988. Reduced corticotropin releasing factor binding sites in the frontal cortex of suicide victims. *Arch Gen Psychiatry*, **45**: 577-579.
- Nutt DJ, French N, Handley S, Hudson A, Husbands S, Jackson H, Jordan S, Lalies MD, Lewis J, Lione L and et al. 1995. Functional studies of specific imidazoline-2 receptor ligands. *Ann N Y Acad Sci*, **763**: 125-139.
- Nutt DJ, Lalies MD, Lione LA and Hudson AL 1997. Noradrenergic mechanisms in the prefrontal cortex. *J Psychopharmacol*, **11**: 163-168.
- O'Neill MF, Osborne DJ, Woodhouse SM and Conway MW 2001. Selective imidazoline I2 ligands do not show antidepressant-like activity in the forced swim test in mice. *J Psychopharmacol*, **15**: 18-22.
- Olmos G, Alemany R, Boronat MA and Garcia-Sevilla JA 1999. Pharmacologic and molecular discrimination of I2-imidazoline receptor subtypes. *Ann N Y Acad Sci*, **881**: 144-160.

- Olmos G, Gabilondo AM, Miralles A, Escriba PV and Garcia-Sevilla JA 1993. Chronic treatment with the monoamine oxidase inhibitors clorgyline and pargyline down-regulates non-adrenoceptor [³H]-idazoxan binding sites in the rat brain. *Br J Pharmacol*, **108**: 597-603.
- Pacak K 2000. Stressor-specific activation of the hypothalamic-pituitary-adrenocortical axis. *Physiol Res*, **49 Suppl 1**: S11-17.
- Pacak K and Palkovits M 2001. Stressor specificity of central neuroendocrine responses: implications for stress-related disorders. *Endocr Rev*, **22**: 502-548.
- Pacheco MA, Stockmeier C, Meltzer HY, Overholser JC, Dilley GE and Jope RS 1996. Alterations in phosphoinositide signaling and G-protein levels in depressed suicide brain. *Brain Res*, **723**: 37-45.
- Pariante CM, Papadopoulos AS, Poon L, Cleare AJ, Checkley SA, English J, Kerwin RW and Lightman S 2004. Four days of citalopram increase suppression of cortisol secretion by prednisolone in healthy volunteers. *Psychopharmacology (Berl)*, **177**: 200-206.
- Parini A, Moudanos CG, Pizzinat N and Lanier SM 1996. The elusive family of imidazoline binding sites. *Trends Pharmacol Sci*, **17**: 13-16.
- Parker CA, Anderson NJ, Robinson ES, Price R, Tyacke RJ, Husbands SM, Dillon MP, Eglen RM, Hudson AL, Nutt DJ, Crump MP and Crosby J 2004. Harmane and harmalan are bioactive components of classical clonidine-displacing substance. *Biochemistry*, **43**: 16385-16392.
- Parker CA, Hudson AL, Nutt DJ, Dillon MP, Eglen RM and Crosby J 2000. Isolation of RP-HPLC pure clonidine-displacing substance from NG108-15 cells. *Eur J Pharmacol*, **387**: 27-30.

- Parker KJ, Schatzberg AF and Lyons DM 2003. Neuroendocrine aspects of hypercortisolism in major depression. *Horm Behav*, **43**: 60-66.
- Paterson LM, Tyacke RJ, Robinson ES, Nutt DJ and Hudson AL 2006. In vitro and in vivo effect of BU99006 (5-isothiocyanato-2-benzofuranyl-2-imidazoline) on I(2) binding in relation to MAO: Evidence for two distinct I(2) binding sites. *Neuropharmacology*.
- Piletz J, Baker R and Halaris A 2008. Platelet imidazoline receptors as state marker of depressive symptomatology. *J Psychiatr Res*, **42**: 41-49.
- Piletz JE, Halaris A and Ernsberger PR 1994. Psychopharmacology of imidazoline and alpha 2-adrenergic receptors: implications for depression. *Crit Rev Neurobiol*, **9**: 29-66.
- Piletz JE, Halaris A, Nelson J, Qu Y and Bari M 1996a. Platelet II-imidazoline binding sites are elevated in depression but not generalized anxiety disorder. *J Psychiatr Res*, **30**: 147-168.
- Piletz JE, Halaris A, Saran A and Marler MR 1991. Desipramine lowers tritiated para-aminoclonidine binding in platelets of depressed patients. *Arch Gen Psychiatry*, **48**: 813-820.
- Piletz JE, Halaris AE, Chikkala D and Qu Y 1996b. Platelet II-imidazoline binding sites are decreased by two dissimilar antidepressant agents in depressed patients. *J Psychiatr Res*, **30**: 169-184.
- Piletz JE, Ivanov TR, Sharp JD, Ernsberger P, Chang CH, Pickard RT, Gold G, Roth B, Zhu H, Jones JC, Baldwin J and Reis DJ 2000. Imidazoline receptor antisera-selected (IRAS) cDNA: cloning and characterization. *DNA Cell Biol*, **19**: 319-329.

Pineda J, Ugedo L and Garcia-Sevilla JA 1993. Stimulatory effects of clonidine, cirazoline and rilmenidine on locus coeruleus noradrenergic neurones: possible involvement of imidazoline-preferring receptors. *Naunyn Schmiedebergs Arch Pharmacol*, **348**: 134-140.

Plotsky PM 1987. Facilitation of immunoreactive corticotropin-releasing factor secretion into the hypophysial-portal circulation after activation of catecholaminergic pathways or central norepinephrine injection. *Endocrinology*, **121**: 924-930.

Popma A, Doreleijers TA, Jansen LM, Van Goozen SH, Van Engeland H and Vermeiren R 2007. The diurnal cortisol cycle in delinquent male adolescents and normal controls. *Neuropsychopharmacology*, **32**: 1622-1628.

Prell GD, Martinelli GP, Holstein GR, Matulic-Adamic J, Watanabe KA, Chan SL, Morgan NG, Haxhiu MA and Ernsberger P 2004. Imidazoleacetic acid-ribotide: an endogenous ligand that stimulates imidazol(in)e receptors. *Proc Natl Acad Sci U S A*, **101**: 13677-13682.

Raddatz R, Parini A and Lanier SM 1995. Imidazoline/guanidinium binding domains on monoamine oxidases. Relationship to subtypes of imidazoline-binding proteins and tissue-specific interaction of imidazoline ligands with monoamine oxidase B. *J Biol Chem*, **270**: 27961-27968.

Reddig PJ, Xu D and Juliano RL 2005. Regulation of p21-activated kinase-independent Rac1 signal transduction by nischarin. *J Biol Chem*, **280**: 30994-31002.

Regunathan S, Meeley MP and Reis DJ 1991. Clonidine-displacing substance from bovine brain binds to imidazoline receptors and releases catecholamines in adrenal chromaffin cells. *Mol Pharmacol*, **40**: 884-888.

Reis DJ and Piletz JE 1997. The imidazoline receptor in control of blood pressure by clonidine and allied drugs. *Am J Physiol*, **273**: R1569-1571.

Reis DJ and Regunathan S 2000. Is agmatine a novel neurotransmitter in brain? *Trends Pharmacol Sci*, **21**: 187-193.

Renouard A, Widdowson PS and Cordi A 1993. [3H]-idazoxan binding to rabbit cerebral cortex recognises multiple imidazoline I₂-type receptors: pharmacological characterization and relationship to monoamine oxidase. *Br J Pharmacol*, **109**: 625-631.

Ribeiro SC, Tandon R, Grunhaus L and Greden JF 1993. The DST as a predictor of outcome in depression: a meta-analysis. *Am J Psychiatry*, **150**: 1618-1629.

Robinson ES, Tyacke RJ, Nutt DJ and Hudson AL 2002. Distribution of [(3)H]BU224, a selective imidazoline I(2) binding site ligand, in rat brain. *Eur J Pharmacol*, **450**: 55-60.

Rommelspacher H, May T and Salewski B 1994. Harman (1-methyl-beta-carboline) is a natural inhibitor of monoamine oxidase type A in rats. *Eur J Pharmacol*, **252**: 51-59.

Rubin RT, Phillips JJ, McCracken JT and Sadow TF 1996. Adrenal gland volume in major depression: relationship to basal and stimulated pituitary-adrenal cortical axis function. *Biol Psychiatry*, **40**: 89-97.

Ruiz-Ortega JA and Ugedo L 1997. The stimulatory effect of clonidine on locus coeruleus neurons of rats with inactivated alpha 2-adrenoceptors: involvement of imidazoline receptors located in the nucleus paragigantocellularis. *Naunyn Schmiedebergs Arch Pharmacol*, **355**: 288-294.

- Samad N, Perveen T, Haider S, Haleem MA and Haleem DJ 2006. Inhibition of restraint-induced neuroendocrine and serotonergic responses by buspirone in rats. *Pharmacol Rep*, **58**: 636-642.
- Sanchez-Blazquez P, Boronat MA, Olmos G, Garcia-Sevilla JA and Garzon J 2000. Activation of I₍₂₎-imidazoline receptors enhances supraspinal morphine analgesia in mice: a model to detect agonist and antagonist activities at these receptors. *Br J Pharmacol*, **130**: 146-52.
- Sano H, Liu SC, Lane WS, Piletz JE and Lienhard GE 2002. Insulin receptor substrate 4 associates with the protein IRAS. *J Biol Chem*, **277**: 19439-19447.
- Saphier D, Farrar GE and Welch JE 1995. Differential inhibition of stress-induced adrenocortical responses by 5-HT1A agonists and by 5-HT2 and 5-HT3 antagonists. *Psychoneuroendocrinology*, **20**: 239-257.
- Saphier D and Feldman S 1989. Catecholaminergic projections to tuberoinfundibular neurones of the paraventricular nucleus: II. Effects of stimulation of the ventral noradrenergic ascending bundle: evidence for cotransmission. *Brain Res Bull*, **23**: 397-404.
- Saphier D and Welch JE 1994. Central stimulation of adrenocortical secretion by 5-hydroxytryptamine1A agonists is mediated by sympathomedullary activation. *J Pharmacol Exp Ther*, **270**: 905-917.
- Sapolsky RM, Romero LM and Munck AU 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocr Rev*, **21**: 55-89.

- Sastre M, Escriba PV, Reis DJ and Garcia-Sevilla JA 1995. Decreased number and immunoreactivity of I₂-imidazoline receptors in the frontal cortex of suicide victims. *Ann N Y Acad Sci*, **763**: 520-522.
- Sautter FJ, Bissette G, Wiley J, Manguno-Mire G, Schoenbachler B, Myers L, Johnson JE, Cerbone A and Malaspina D 2003. Corticotropin-releasing factor in posttraumatic stress disorder (PTSD) with secondary psychotic symptoms, nonpsychotic PTSD, and healthy control subjects. *Biol Psychiatry*, **54**: 1382-1388.
- Schelling G, Kilger E, Rozendaal B, de Quervain DJ, Biegel J, Dagge A, Rothenhausler HB, Krauseneck T, Nollert G and Kapfhammer HP 2004. Stress doses of hydrocortisone, traumatic memories, and symptoms of posttraumatic stress disorder in patients after cardiac surgery: a randomized study. *Biol Psychiatry*, **55**: 627-633.
- Schmidt U, Frerick, H, Kraft, K, Schenk, N, Löw-Kröger, A 1992. Hypertension: A possible risk in road traffic. *J Cardiovasc Pharmacol.*, **20 Suppl**: S50-S56.
- Scott LV and Dinan TG 1998. Vasopressin and the regulation of hypothalamic-pituitary-adrenal axis function: implications for the pathophysiology of depression. *Life Sci*, **62**: 1985-1998.
- Separovic D, Kester M and Ernsberger P 1996. Coupling of I₁-imidazoline receptors to diacylglyceride accumulation in PC12 rat pheochromocytoma cells. *Mol Pharmacol*, **49**: 668-675.
- Separovic D, Kester M, Haxhiu MA and Ernsberger P 1997. Activation of phosphatidylcholine-selective phospholipase C by I₁-imidazoline receptors in PC12 cells and rostral ventrolateral medulla. *Brain Res*, **749**: 335-339.

Silverman AJ, Hou-Yu A and Chen WP 1989. Corticotropin-releasing factor synapses within the paraventricular nucleus of the hypothalamus. *Neuroendocrinology*, **49**: 291-299.

Smoller JW, Yamaki LH, Fagerness JA, Biederman J, Racette S, Laird NM, Kagan J, Snidman N, Faraone SV, Hirshfeld-Becker D, Tsuang MT, Slauenhaft SA, Rosenbaum JF and Sklar PB 2005. The corticotropin-releasing hormone gene and behavioral inhibition in children at risk for panic disorder. *Biol Psychiatry*, **57**: 1485-1492.

Soravia LM, Heinrichs M, Aerni A, Maroni C, Schelling G, Ehlert U, Roozendaal B and de Quervain DJ 2006. Glucocorticoids reduce phobic fear in humans. *Proc Natl Acad Sci U S A*, **103**: 5585-5590.

Squires PE, Hills CE, Rogers GJ, Garland P, Farley SR and Morgan NG 2004. The putative imidazoline receptor agonist, harmine, promotes intracellular calcium mobilisation in pancreatic beta-cells. *Eur J Pharmacol*, **501**: 31-39.

Sun Z, Chang CH and Ernsberger P 2007. Identification of IRAS/Nischarin as an I1-imidazoline receptor in PC12 rat pheochromocytoma cells. *J Neurochem*, **101**: 99-108.

Swanson LW, Sawchenko PE, Rivier J and Vale WW 1983. Organization of ovine corticotropin-releasing factor immunoreactive cells and fibers in the rat brain: an immunohistochemical study. *Neuroendocrinology*, **36**: 165-186.

Szabo B 2002. Imidazoline antihypertensive drugs: a critical review on their mechanism of action. *Pharmacol Ther*, **93**: 1-35.

Szabo B and Urban R 1995. Mechanism of sympathoinhibition by imidazolines. *Ann N Y Acad Sci*, **763**: 552-565.

- Takada K, Hayashi Y, Kamibayashi T, Mammoto T, Yamatodani A, Kitamura S and Yoshiya I 1997. The involvement of pertussis toxin-sensitive G proteins in the post receptor mechanism of central I1-imidazoline receptors. *Br J Pharmacol*, **120**: 1575-1581.
- Talalaenko AN, Krivobok GK, Pankrat'ev DV and Goncharenko NV 2006. Neurochemical mechanisms of the dorsal pallidum in the antiaversive effects of anxiolytics in various models of anxiety. *Neurosci Behav Physiol*, **36**: 749-754.
- Teixeira de Castro RR, Tibirica E, de Oliveira MA, Moreira PB, Catelli MF, Rocha NN and Nobrega AC 2006. Reduced hemodynamic responses to physical and mental stress under low-dose rilmenidine in healthy subjects. *Cardiovasc Drugs Ther*, **20**: 129-134.
- Tesson F, Limon-Boulez I, Urban P, Puype M, Vandekerckhove J, Coupry I, Pompon D and Parini A 1995. Localization of I2-imidazoline binding sites on monoamine oxidases. *J Biol Chem*, **270**: 9856-9861.
- Thomas B and Prell GD 1995. Imidazoleacetic acid, a gamma-aminobutyric acid receptor agonist, can be formed in rat brain by oxidation of histamine. *J Neurochem*, **65**: 818-826.
- Thomson F and Craighead M 2007. Innovative Approaches for the Treatment of Depression: Targeting the HPA Axis. *Neurochem Res*.
- Tibirica E, Mermet C, Feldman J, Gonon F and Bousquet P 1989. Correlation between the inhibitory effect on catecholaminergic ventrolateral medullary neurons and the hypotension evoked by clonidine: a voltammetric approach. *J Pharmacol Exp Ther*, **250**: 642-647.

- Touiki K, Rat P, Molimard R, Chait A and de Beaurepaire R 2005. Harmane inhibits serotonergic dorsal raphe neurons in the rat. *Psychopharmacology (Berl)*, **182**: 562-569.
- Tunnicliff G 1998. Pharmacology and function of imidazole 4-acetic acid in brain. *Gen Pharmacol*, **31**: 503-509.
- Tyacke RJ, Robinson ES, Nutt DJ and Hudson AL 2002. 5-Isothiocyanato-2-benzofuranyl-2-imidazoline (BU99006) an irreversible imidazoline(2) binding site ligand: in vitro and in vivo characterisation in rat brain. *Neuropharmacology*, **43**: 75-83.
- Ugedo L, Pineda J, Martin-Ruiz R, Ruiz-Ortega JA and Artigas F 1999. Imidazoline-induced inhibition of firing rate of 5-HT neurons in rat dorsal raphe by modulation of extracellular 5-HT levels. *Ann N Y Acad Sci*, **881**: 365-368.
- Ugedo L, Pineda J, Ruiz-Ortega JA and Martin-Ruiz R 1998. Stimulation of locus coeruleus neurons by non-I1/I2-type imidazoline receptors: an in vivo and in vitro electrophysiological study. *Br J Pharmacol*, **125**: 1685-1694.
- Valentino RJ and Curtis AL 1991. Pharmacology of locus coeruleus spontaneous and sensory-evoked activity. *Prog Brain Res*, **88**: 249-256.
- Van Den Eede F, Van Broeckhoven C and Claes SJ 2005. Corticotropin-releasing factor-binding protein, stress and major depression. *Ageing Res Rev*, **4**: 213-239.
- Wang LG, Gao L, Wang W, Yuan WJ and Wang WZ 2007. Sympathoexcitation of moxonidine in the caudal ventrolateral medulla is dependent on I1-imidazoline receptors in anesthetized rats. *Neurosci Lett*, **426**: 91-96.
- Welch JE, Farrar GE, Dunn AJ and Saphier D 1993. Central 5-HT1A receptors inhibit adrenocortical secretion. *Neuroendocrinology*, **57**: 272-281.

- Welch JE and Saphier D 1994. Central and peripheral mechanisms in the stimulation of adrenocortical secretion by the 5-hydroxytryptamine2 agonist, (+)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane. *J Pharmacol Exp Ther*, **270**: 918-928.
- Wenzel RR, Mitchell A, Siffert W, Buhrmann S, Philipp T and Schafers RF 2004. The H1-imidazoline agonist moxonidine decreases sympathetic tone under physical and mental stress. *Br J Clin Pharmacol*, **57**: 545-551.
- Wolkowitz OM, Reus VI, Chan T, Manfredi F, Raum W, Johnson R and Canick J 1999. Antiglucocorticoid treatment of depression: double-blind ketoconazole. *Biol Psychiatry*, **45**: 1070-1074.
- Yang YL, Chao PK and Lu KT 2006. Systemic and intra-amygdala administration of glucocorticoid agonist and antagonist modulate extinction of conditioned fear. *Neuropsychopharmacology*, **31**: 912-924.
- Yehuda R, Yang RK, Buchsbaum MS and Golier JA 2006. Alterations in cortisol negative feedback inhibition as examined using the ACTH response to cortisol administration in PTSD. *Psychoneuroendocrinology*, **31**: 447-451.
- Yu YW, Tsai SJ, Hong CJ, Chen TJ, Chen MC and Yang CW 2005. Association study of a monoamine oxidase a gene promoter polymorphism with major depressive disorder and antidepressant response. *Neuropsychopharmacology*, **30**: 1719-1723.
- Zacharko RM and Anisman H 1991. Stressor-induced anhedonia in the mesocorticolimbic system. *Neurosci Biobehav Rev*, **15**: 391-405.
- Zeidan MP, Zomkowski AD, Rosa AO, Rodrigues AL and Gabilan NH 2007. Evidence for imidazoline receptors involvement in the agmatine antidepressant-like effect in the forced swimming test. *Eur J Pharmacol*, **565**: 125-131.

Zhang J and Abdel-Rahman AA 2005. Mitogen-activated protein kinase phosphorylation in the rostral ventrolateral medulla plays a key role in imidazoline (I1)-receptor-mediated hypotension. *J Pharmacol Exp Ther*, **314**: 945-952.

Zhang J and Abdel-Rahman AA 2006. Nischarin as a functional imidazoline (I1) receptor. *FEBS Lett*, **580**: 3070-3074.

Zhang J, El-Mas MM and Abdel-Rahman AA 2001. Imidazoline I(1) receptor-induced activation of phosphatidylcholine-specific phospholipase C elicits mitogen-activated protein kinase phosphorylation in PC12 cells. *Eur J Pharmacol*, **415**: 117-125.

Zhu H, Halaris A, Madakasira S, Pazzaglia P, Goldman N, DeVane CL, Andrew M, Reis D and Piletz JE 1999. Effect of bupropion on immunodensity of putative imidazoline receptors on platelets of depressed patients. *J Psychiatr Res*, **33**: 323-333.

Zhu H, Halaris A and Piletz JE 1997a. Chronic imipramine treatment downregulates IR1-imidazoline receptors in rat brainstem. *Life Sci*, **61**: 1973-1983.

Zhu H, Hayes J, Chen M, Baldwin J and Piletz JE 2003. Relationship between platelet imidazoline receptor-binding peptides and candidate imidazoline-1 receptor, IRAS. *Ann NY Acad Sci*, **1009**: 439-446.

Zhu H, Paul IA, McNamara M, Redmond A, Nowak G and Piletz JE 1997b. Chronic imipramine treatment upregulates IR2-imidazoline receptive sites in rat brain. *Neurochem Int*, **30**: 101-107.

Zobel AW, Nickel T, Kunzel HE, Ackl N, Sonntag A, Ising M and Holsboer F 2000. Effects of the high-affinity corticotropin-releasing hormone receptor 1 antagonist

R121919 in major depression: the first 20 patients treated. *J Psychiatr Res*, **34**: 171-181.

Zobel AW, Nickel T, Sonntag A, Uhr M, Holsboer F and Ising M 2001. Cortisol response in the combined dexamethasone/CRH test as predictor of relapse in patients with remitted depression. a prospective study. *J Psychiatr Res*, **35**: 83-94.

Zomkowski AD, Hammes L, Lin J, Calixto JB, Santos AR and Rodrigues AL 2002. Agmatine produces antidepressant-like effects in two models of depression in mice. *Neuroreport*, **13**: 387-391.

Table 1 Modulation of brain monoamine activity by IBS ligands* in rodents *in vivo*

Ligand	Pharmacology	Action	Brain region	Reference
Harmane	Endogenous	↑ noradrenergic firing ↓ serotonergic firing	Locus coeruleus Dorsal raphe	Ruiz-Durantez <i>et al.</i> , 2001 Touiki <i>et al.</i> , 2005; Ugedo <i>et al.</i> , 1998
		↑ serotonin ↑ serotonin & dopamine spontaneous neuronal activity	Hippocampus Nucleus accumbans Nucleus accumbans	Adell <i>et al.</i> , 1996 Baum <i>et al.</i> , 1996 Ergene and Schoener 1993
BU224	I ₂ ligand	↑ noradrenaline ↑ noradrenaline	PVN	Finn <i>et al.</i> , 2002
		↓ noradrenaline turnover ↓ serotonin turnover	Frontal Cortex and hippocampus Frontal Cortex Hippocampus and hypothalamus	Hudson <i>et al.</i> , 1999; Nutt <i>et al.</i> , 1997 Finn <i>et al.</i> , 2003 Finn <i>et al.</i> , 2003
2-BFI	I ₂ ligand	↑ noradrenaline	Frontal cortex and hippocampus	Nutt <i>et al.</i> , 1995
		↑ noradrenergic firing ↑ noradrenaline	Locus coeruleus Striatum	Ugedo <i>et al.</i> , 1998 Sastre-Coll <i>et al.</i> , 2001
BU98008	I ₂ ligand	↑ noradrenaline & ↑ dopamine overflow	Frontal Cortex	Abu Ghazaleh <i>et al.</i> , 2007
Clonidine with EEDQ pretreatment	I ₁ /I ₂ ligand	↑ noradrenergic firing ↓ DOPA, 5-HTP synthesis	Locus coeruleus Cerebral cortex, Hippocampus Striatum	Pineda <i>et al.</i> , 1993 Sastre-Coll <i>et al.</i> , 1999
			Cerebral cortex Hippocampus	Sastre-Coll <i>et al.</i> , 1999
Moxonidine	I ₁ agonist	↓ DOPA, 5-HTP synthesis	Cerebral cortex Hippocampus	Sastre-Coll <i>et al.</i> , 1999
Rilmenidine	I ₁ agonist	↑ neuronal activation ↓ DOPA synthesis ↓ 5-HTP synthesis	Brain Striatum Cerebral cortex	Pineda <i>et al.</i> , 1993 Sastre-Coll <i>et al.</i> , 1999
Cirazoline	I ₁ agonist	↑ neuronal activation	Brain	Pineda <i>et al.</i> , 1993
Efaroxan	I ₁ antagonist	↓ 5-HT synthesis	Hippocampus & Cerebral cortex	Sastre-Coll <i>et al.</i> , 1999

*Footnote to table 1:

Increased understanding of the pharmacology and signal transduction mechanisms associated with I₁ IBS permits the designation of pharmacological activity (agonist/antagonist) to the majority of I₁ IBS selective compounds. In contrast, I₂ IBS pharmacology and signalling mechanisms are less well understood and it is therefore more difficult to assign pharmacological properties to I₂ compounds. However, similarities between the effects of I₂ ligands BU224, 2BFI and BU99066 with those of the endogenous ligand harmane, together with supporting evidence from (Diaz *et al.*, 1997; Sanchez-Blazquez *et al.*, 2000), suggest that these ligands may be agonistic in nature.

Ligand	Pharmacology	Species	Action on HPA axis	References
BU224	I ₂ ligand	Rat Rat Human	↑ plasma ACTH ↑ plasma corticosterone ↑ plasma ACTH & cortisol	Finn <i>et al.</i> , 2003 Finn <i>et al.</i> , 2004, 2002 Munoz-Hoyos <i>et al.</i> , 2000
Harmaline	Endogenous	Cat	↑ CRF expression	Cummings <i>et al.</i> , 1994
Idazoxan	I ₂ ligand	Rat Human	↑ plasma corticosterone ↓ diurnal cortisol tone	Finn <i>et al.</i> , 2004 Glue <i>et al.</i> , 1992
Clonidine	I ₁ ligand	Horse Human	↓ ACTH secretion ↓ plasma ACTH & cortisol	Alexander <i>et al.</i> , 2000 Munoz-Hoyos <i>et al.</i> , 2000

Table 2 Modulation of stress hormones by IBS ligands

Figure legends

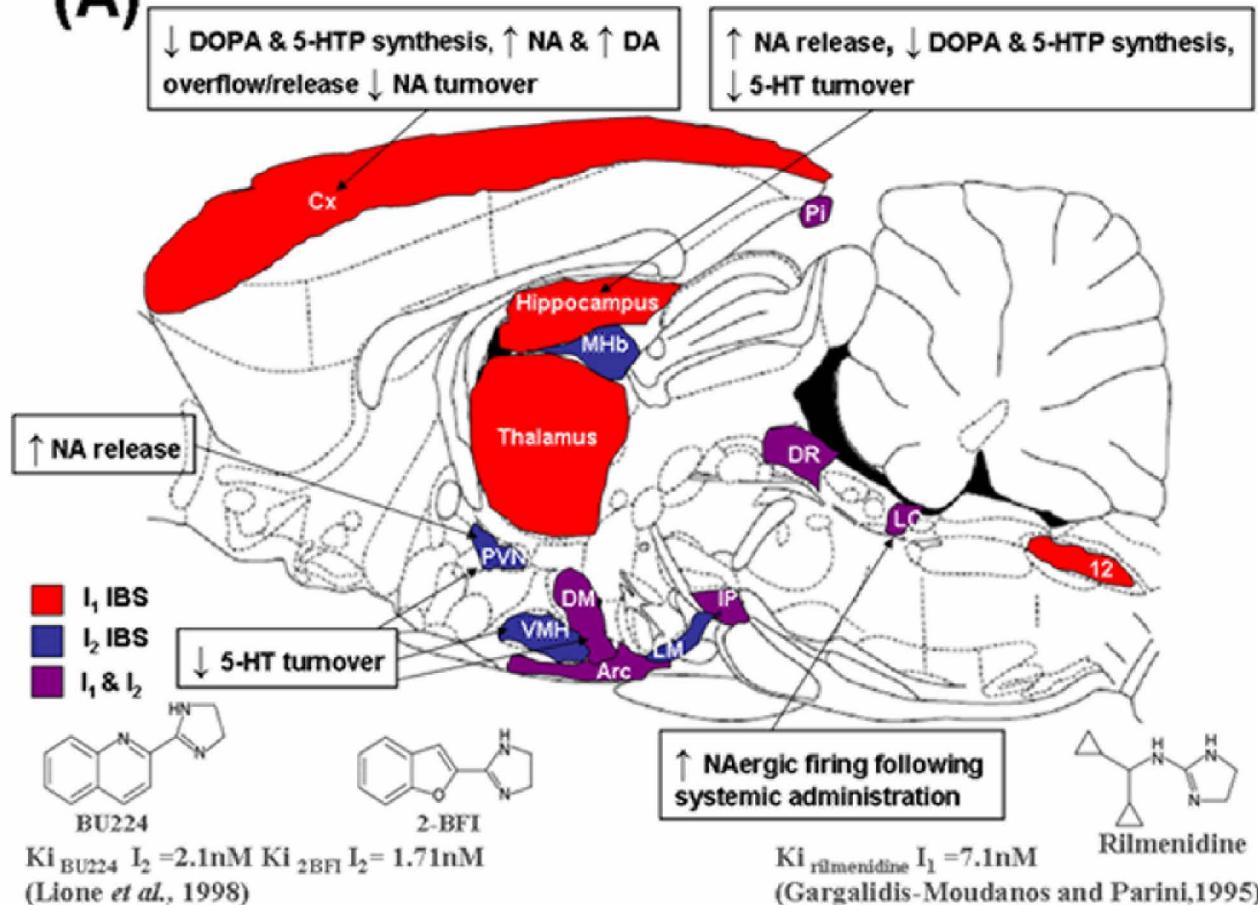
Figure 1: **(A)** Schematic depicting expression of imidazoline binding sites in the rat brain as determined by receptor autoradiography using [³H]BU224 (I₂ selective ligand), [³H]2BFI (I₂ selective ligand) and [³H] rilmenidine (I₁ selective ligand). The regions highlighted have been shown to express a moderate to high density of imidazoline binding sites (King *et al.*, 1995; Lione *et al.*, 1998; Robinson *et al.*, 2002; Tyacke *et al.*, 2002). Figure also summarises the effects of imidazoline binding site activation on neuronal firing and terminal release of monoamines in these regions. I₁ (red) and I₂ (blue) binding sites are colocalised (purple) in some brain regions. **(B)** Schematic depicting [³H] harmane binding (green) in discrete rat brain regions and the effects of harmane administration on neuronal firing and terminal release of monoamines in these regions. Abbreviations: AP area postrema; Arc arcuate nucleus; CG central grey; Cx Cortex; DR dorsal raphe; DM dorsomedial hypothalamic nucleus; Fr frontal cortex; IP interpeduncular nucleus; LC locus coeruleus; LM lateral mammillary nucleus; LS lateral septal nucleus; MHb medial habenular nucleus; Occ occipital cortex; Pi pineal gland; PVN paraventricular nucleus (hypothalamus); Sol nucleus of the solitary tract; SuG superficial gray layer of the superior colliculus; VMH ventromedial hypothalamic nucleus; 12 hypoglossal nucleus.

Figure 2 Effect of systemic administration of the selective I₂ IBS ligand, BU224, on (a) extracellular levels of NA in the PVN region and (b) plasma corticosterone levels in control rats and rats with adjuvant-induced arthritis (AA). Values are expressed as

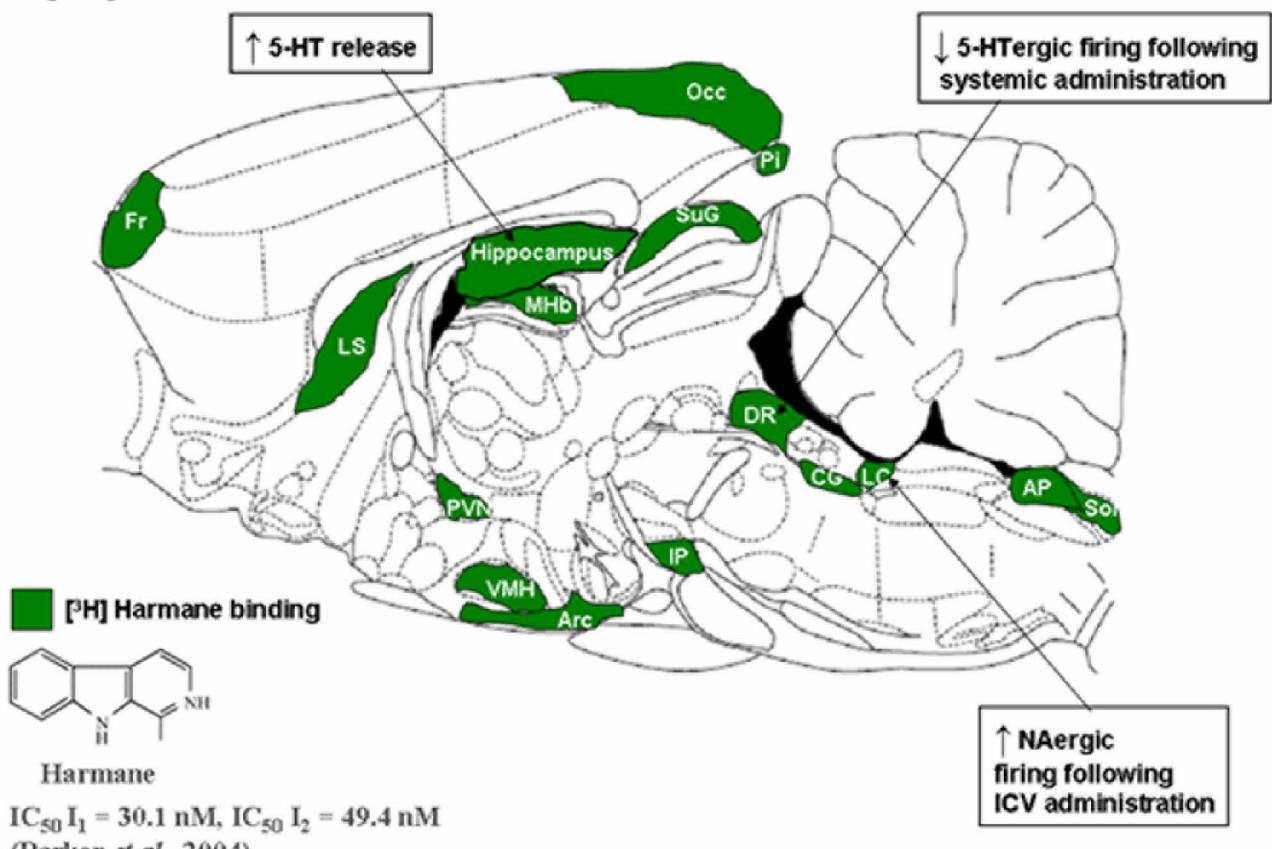
mean \pm s.e. mean. **P<0.01, *P<0.05 comparing post-injection levels with basal levels in the Con+BU224 group; $^+P<0.05$, $^{++}P<0.01$ comparing post-injection levels with basal levels in the AA+BU224 group; $^{\$}P<0.05$ comparing AA+BU224 with Con+BU224 group at 60 min. Control (Con); Saline (Sal). Reproduced with permission from Finn *et al.*, 2002.

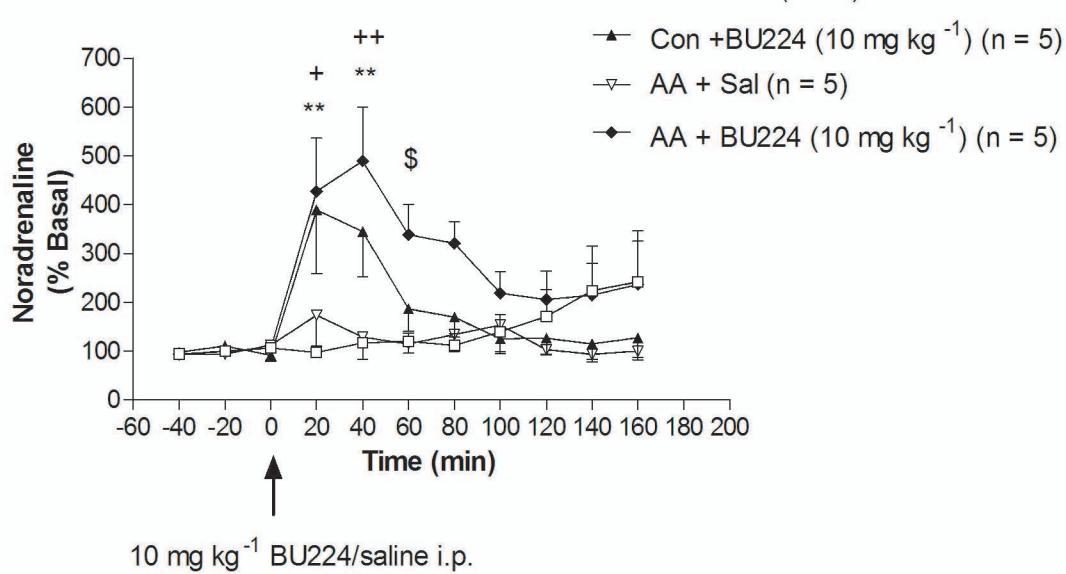
Figure 3 Effects of the selective I₂ binding site ligand BU224 (10 mg/kg, i.p.) on plasma corticosterone levels in control and restraint stressed rats. Results are expressed as mean \pm SEM ($n = 5\text{--}7$). ** $p < 0.01$ and $^{++}p < 0.01$ compared with saline-treated controls at 30 and 60 min, respectively. $^{\ddagger}p < 0.05$ and $^{\dagger}p < 0.01$ compared with BU224-treated controls at 30 and 60 min, respectively. $^{\$\$}P < 0.05$ compared with saline-treated restrained rats at 60 min. Saline + Control (Sal + Con); BU224 + Control (BU + Con); Saline + Restraint (Sal + Res); BU224 + Restraint (BU + Res). Data reproduced with permission from Finn *et al.*, 2004.

(A)



(B)



(a)**(b)**