REVIEW

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

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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 serotoninergic 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 adrenocorticotropic 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 predicative 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; Jolkkonen *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.

Serotoninergic modulation

Serotoninergic (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 solitarii (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: $I_{1,}$ I_2 and I_3 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).

I_1 binding sites

I_1 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). I_1 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/nisacharin 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 elelments of Rac1 signaling pathways (Reddig *et al.*, 2005). Studies investigating I_1 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 I_1 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 I_1 IBS from neuronal α_2-adrenoceptors (Separovic *et al.*, 1997).

I_1 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 I_1 IBS are localized in the RVLM, a region involved in cardiovascular activity. Evidence supporting a role for I_1 IBS in sympathoinhibition comes from work demonstrating that drugs devoid of affinity for α_2-adrenoceptors, but with affinity for I_1 IBS, lower blood pressure when injected into the RVLM (Bousquet *et al.*, 1984; Laubie *et al.*, 1985; Tibirica *et al.*, 1989).

Moreover, I_1 IBS antagonists block the cardiovascular effects of I_1 IBS agonists more effectively than pure α_2-adrenoceptor antagonists (Wang *et al.*, 2007). I_1 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 I_1 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.

I_2 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). I_2 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). I_2 IBS are widely distributed in the central nervous system (CNS) and peripheral tissues. [^{3}H]idazoxan, and more recently [^{3}H]2-BFI and [^{3}H]BU224, have proven to be important tools for elucidating the distribution of I_2 IBS. All three ligands possess high affinity for the I_2 IBS and the latter two possess a high degree of selectivity for I_2 IBS over α_2-adrenoceptors and I_1 IBS (Dardonville and Rozas, 2004). Using receptor autoradiography, the highest densities of [^{3}H]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 [^{3}H]BU224 and [^{3}H]idazoxan (Robinson *et al.*, 2002). I_2 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 I_2 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 I_2 IBS during purification processes (Tesson *et al.*, 1995), suggests a strong relationship between the I_2 IBS and MAO.

[^{3}H]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 Ki values of 5, 69, 15, and 48 nM, respectively (Kim *et al.*, 1997). However, residual [^{3}H]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 I_2 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 I_2 IBS

ligand BU99006 result in a decrease in [^{3}H]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 I_2 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 I_2 IBS in the brain (Olmos *et al.*, 1993). These findings implicate I_2 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.

I_3 binding site

The more recently identified I_3 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 I_3 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 I_1 IBS and I_2 IBS. Harmane significantly reduces blood pressure by activating I_1 IBS (Musgrave and Badoer, 2000), modulates monoamine oxidases via the I_2 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 I_3 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 I_1 IBS in adrenal tissue and promote the release of arachidonic acid from PC12 cells (Prell *et al.*, 2004). However, as a potential I_1 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 I_3R 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 I_3R-mediated response (Chan *et al.*, 1997; Eglen *et al.*, 1998; Morgan *et al.*, 1999). I_3 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 I_3R agonists. Interestingly, IAA-RP was more potent than the prototypical I_3 agonist /I_1 antagonist efaroxan EC_{50} =30-50 nM (Prell *et al.*, 2004) versus 100 mM (Chan and Morgan, 1990; Chan *et al.*, 1997). Moreover, efaroxan 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 ± 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 I_1 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 serotoninergic 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 resperine and p-chloro-phenylalanine 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 I_2 IBS in the modulation of brain monoamines has also been demonstrated. Both central and systemic administration of the selective I_2 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 I_2 IBS ligands is not mediated by I_1 or I_2 IBS, despite the high density of I_2 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 D_2, 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, I_2 IBS ligands increase extracellular levels of serotonin in the hippocampus and LC in the rat (Adell *et al.*, 1996; Ugedo *et al.*, 1999). I_2 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 I_2 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 I_2 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 that 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) I_2 IBS ligands stimulate HPA axis activity by increasing release of NA in the PVN and (b) the noradrenergic response to an I_2 IBS ligand is enhanced in the PVN of rats with adjuvant-induced arthritis. This indicates that I_2 IBS is an important pharmacological target as its activation facilitates NA release under basal and chronic stress conditions.

[Insert figure 2 here]

Selective I_2 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, I_2 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 I_2 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 olivary 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 I_1 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 I_2 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 I_1 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 I_1 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 I_2 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 I_2 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 I_1 and I_2 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 efaroxan, idazoxan and antazoline (a ligand with high affinity for the I_2 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 I_1 and I_2 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 (Marti 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 I_2 IBS expression on platelet membranes is significantly reduced in platelets of depressed patients (Piletz *et al.*, 1994). Furthermore, I_2 IBS expression is altered in brain tissue. I_2 IBS are reduced by 40% in the frontal cortex of suicide victims (Sastre *et al.*, 1995).

Since the association between MAO and I_2 IBS is well established, studies investigating the correlation between MAO-B and suicidal behaviour are of particular relevance to the role of I_2-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 I_1 IBS densities have also been reported. Radioligand binding density (B_{max}) of I_1 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 I_1 IBS as detected by [^{125}I]*p*-iodoclonidine (Zhu *et al.*, 2003). Other bands have been identified and correlated with the I_1 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 I_1 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 $G\alpha_{q/11}$, $G\alpha_{i2}$ and Gβ 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 [^{3}H] 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 I_1 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 I_1 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 I_1 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 I_1 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 I_1 and I_2 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 I_2 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.

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

Ligand	Pharmacology	Action	Brain region	Reference
Harmane	Endogenous	↑ noradrenerigic firing ↓ serotoninerigic firing	Locus coeruleus Dorsal raphe	Ruiz-Durantez *et al.*, 2001 Touiki *et al.*, 2005; Ugedo *et al.*, 1998
		↑ serotonin	Hippocampus	Adell *et al.*, 1996
		↑ serotonin & dopamine	Nucleus accumbans	Baum *et al.*, 1996
		spontaneous neuronal activity	Nucleus accumbans	Ergene and Schoener 1993
BU224	I_2 ligand	↑ noradrenaline	PVN	Finn *et al.*, 2002
		↑ noradrenaline	Frontal Cortex and hippocampus	Hudson *et al.*, 1999; Nutt *et al.*, 1997
		↓ noradrenaline turnover	Frontal Cortex	Finn *et al.*, 2003
		↓ serotonin turnover	Hippocampus and hypothalamus	Finn *et al.*, 2003
2-BFI	I_2 ligand	↑ noradrenaline	Frontal cortex and hippocampus	Nutt *et al.*, 1995
		↑ noradrenergic firing	Locus coeruleus	Ugedo *et al.*, 1998
		↑ noradrenaline	Striatum	Sastre-Coll *et al.*, 2001
BU98008	I_2 ligand	↑ noradrenaline & ↑ dopamine overflow	Frontal Cortex	Abu Ghazaleh *et al.*, 2007
Clonidine with EEDQ pretreatment	I_1/I_2 ligand	↑ noradrenergic firing ↓ DOPA, 5-HTP synthesis	Locus coeruleus Cerebral cortex, Hippocampus Striatum	Pineda *et al.*, 1993 Sastre-Coll *et al.*, 1999
Moxonidine	I_1 agonist	↓ DOPA, 5-HTP synthesis	Cerebral cortex Hippocampus	Sastre-Coll *et al.*, 1999
Rilmenidine	I_1 agonist	↑ neuronal activation ↓ DOPA synthesis ↓ 5-HTP synthesis	Brain Striatum Cerebral cortex	Pineda *et al.*, 1993 Sastre-Coll *et al.*, 1999
Cirazoline	I_1 agonist	↑ neuronal activation	Brain	Pineda *et al.*, 1993
Efaroxan	I_1 antagonist	↓ 5-HT synthesis	Hippocampus & Cerebral cortex	Sastre-Coll *et al.*, 1999

*Footnote to table 1:

Increased understanding of the pharmacology and signal transduction mechanisms associated with I_1 IBS permits the designation of pharmacological activity (agonist/antagonist) to the majority of I_1 IBS selective compounds. In contrast, I_2 IBS pharmacology and signalling mechanisms are less well understood and it is therefore more difficult to assign pharmacological properties to I_2 compounds. However, similarities between the effects of I_2 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 *et al.*, 2003 Finn *et al.*, 2004, 2002 Munoz-Hoyos *et al.*, 2000
Harmaline	Endogenous	Cat	↑ CRF expression	Cummings *et al.*, 1994
Idazoxan	I_2 ligand	Rat Human	↑ plasma corticosterone ↓ diurnal cortisol tone	Finn *et al.*, 2004 Glue *et al.*, 1992
Clonidine	I_1 ligand	Horse Human	↓ ACTH secretion ↓ plasma ACTH & cortisol	Alexander *et al.*, 2000 Munoz-Hoyos *et al.*, 2000

Table 2 Modulation of stress hormones by IBS ligands

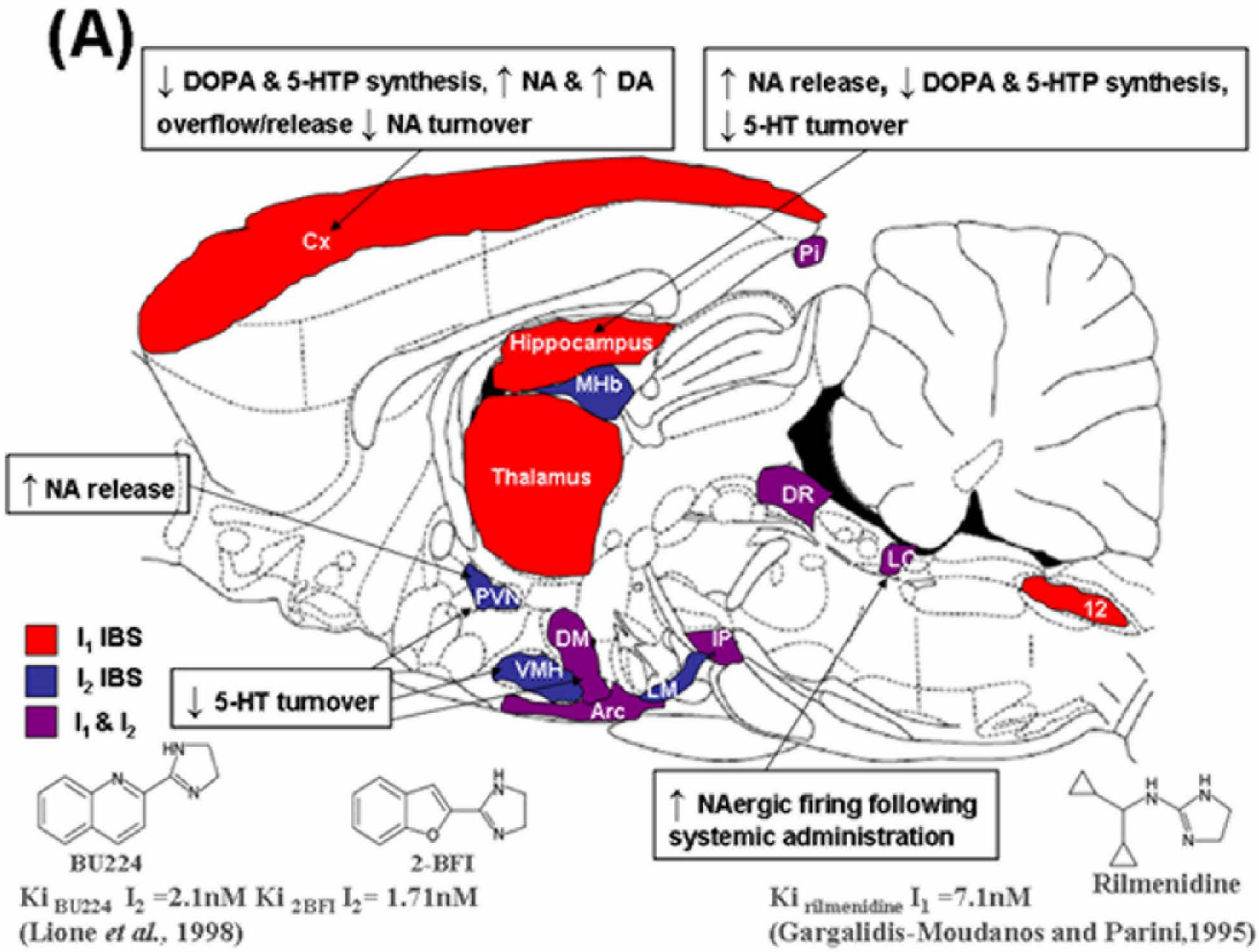
Figure legends

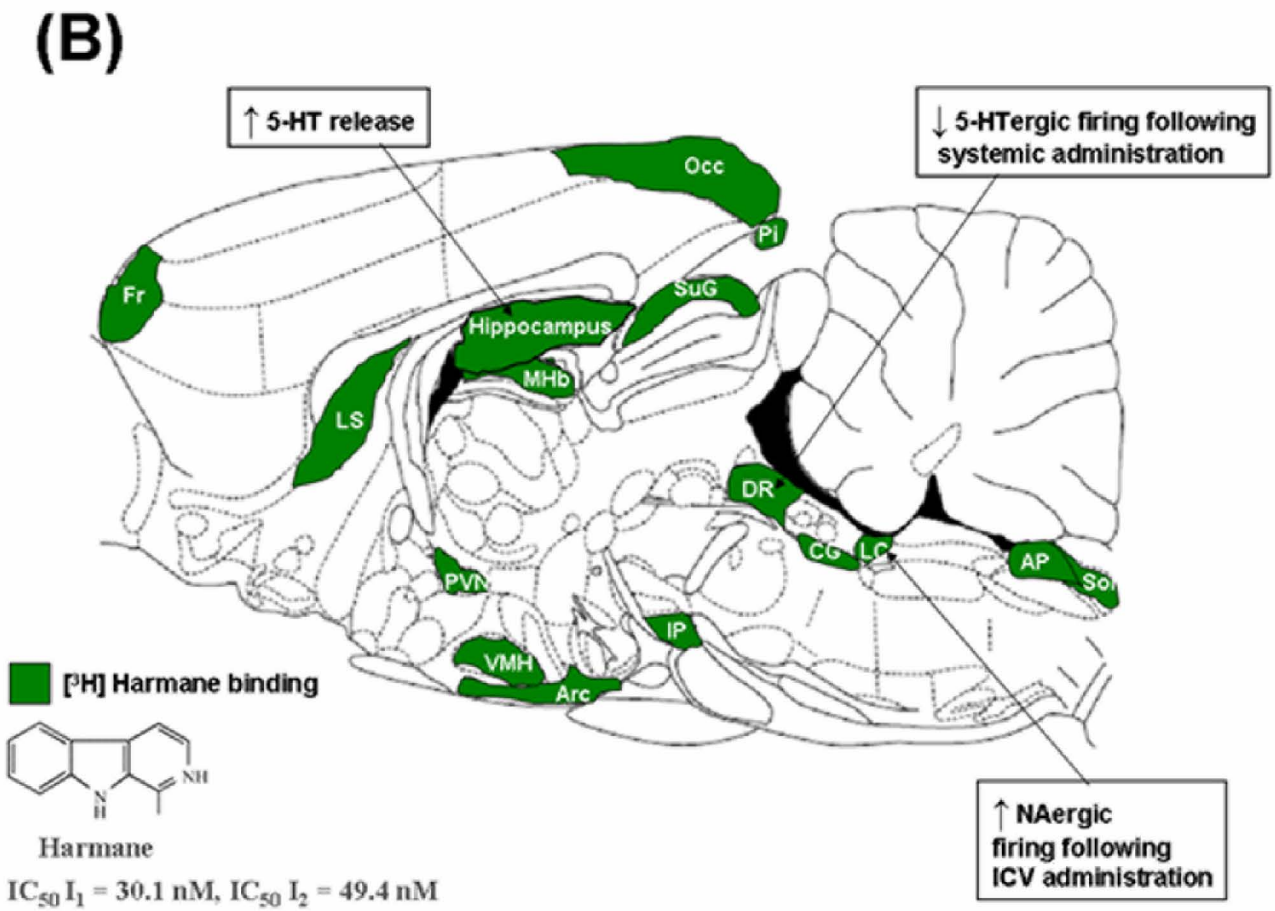
Figure 1: **(A)** Schematic depicting expression of imidazoline binding sites in the rat brain as determined by receptor autoradiography using [^{3}H]BU224 (I_2 selective ligand), [^{3}H]2BFI (I_2 selective ligand) and [^{3}H] rilmenidine (I_1 selective ligand). The regions highlighted have been shown 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. I_1 (red) and I_2 (blue) binding sites are colocalised (purple) in some brain regions. **(B)** Schematic depicting [^{3}H] 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.

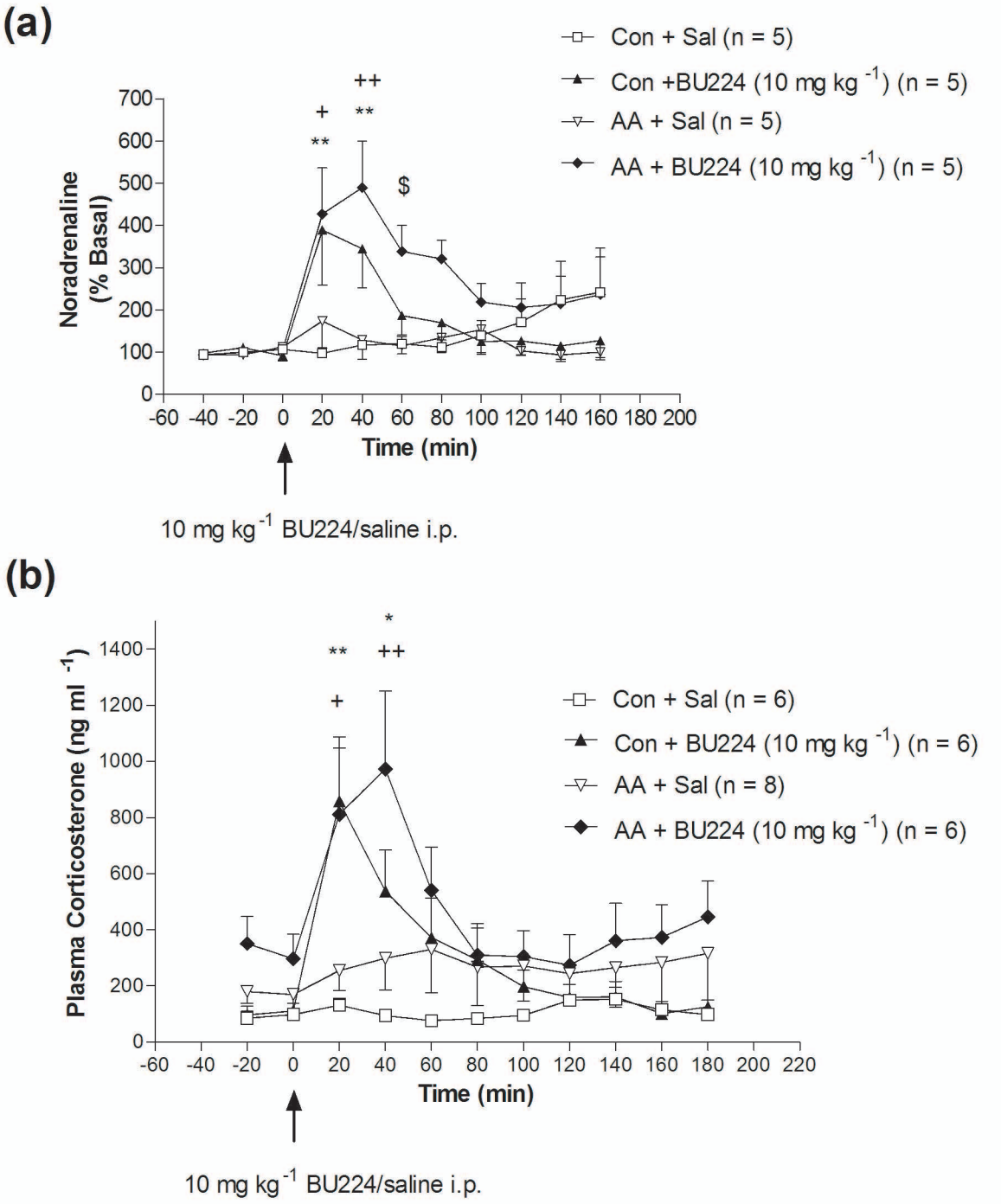
Figure 2 Effect of systemic administration of the selective I_2 IBS ligand, BU224, on (a) extracellular levels of NA in the PVN region and (b) plasma corticosterone levels in control rats and rats with adjuvant-induced arthritis (AA). Values are expressed as

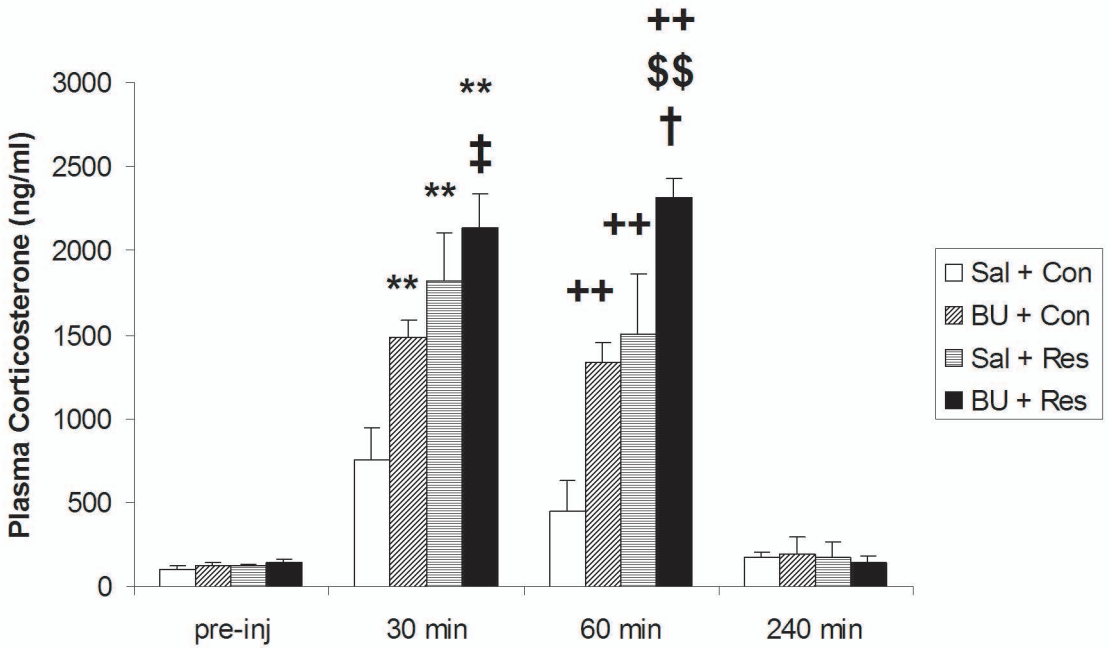
mean±s.e. mean. **P<0.01, *P<0.05 comparing post-injection levels with basal levels in the Con+BU224 group; $^{+}$P<0.05, $^{++}$P<0.01 comparing post-injection levels with basal levels in the AA+BU224 group; $^{\$}$P<0.05 comparing AA+BU224 with Con+BU224 group at 60 min. Control (Con); Saline (Sal).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

Figure 3 Effects of the selective I_2 binding site ligand BU224 (10 mg/kg, i.p.) on plasma corticosterone levels in control and restraint stressed rats. Results are expressed as mean ± SEM (n = 5–7). **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)
↓ DOPA & 5-HTP synthesis, ↑ NA & ↑ DA overflow/release ↓ NA turnover
↑ NA release, ↓ DOPA & 5-HTP synthesis, ↓ 5-HT turnover
Cx
Pi
Hippocampus
MHb
Thalamus
↑ NA release
DR
LC
PVN
12
DM
IP
VMH
LM
Arc
I1 IBS
I2 IBS
I1 & I2
↓ 5-HT turnover
↑ NAergic firing following systemic administration
BU224
2-BFI
Rilmenidine
Ki BU224 I2 =2.1nM Ki 2BFI I2= 1.71nM
(Lione et al., 1998)
Ki rilmenidine I1 =7.1nM
(Gargalidis-Moudanos and Parini,1995)


(B)
↑ 5-HT release
↓ 5-HTergic firing following systemic administration
Occ
Pi
Fr
SuG
Hippocampus
MHb
LS
DR
CG
LC
AP
So
PVN
IP
VMH
Arc
[3H] Harmane binding
Harmane
↑ NAergic firing following ICV administration
IC50 I1 = 30.1 nM, IC50 I2 = 49.4 nM
(Parker et al., 2004)


(a)
Con + Sal (n = 5)
Con +BU224 (10 mg kg -1) (n = 5)
AA + Sal (n = 5)
AA + BU224 (10 mg kg -1) (n = 5)
Noradrenaline (% Basal)
Time (min)
10 mg kg -1 BU224/saline i.p.
(b)
Con + Sal (n = 6)
Con + BU224 (10 mg kg -1) (n = 6)
AA + Sal (n = 8)
AA + BU224 (10 mg kg -1) (n = 6)
Plasma Corticosterone (ng ml -1)
Time (min)
10 mg kg -1 BU224/saline i.p.


Plasma Corticosterone (ng/ml)
3000
2500
2000
1500
1000
500
0
pre-inj
30 min
60 min
240 min
**
**
**
‡
++
++
++
$$
†
Sal + Con
BU + Con
Sal + Res
BU + Res