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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 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 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.

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 α1-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 $\alpha_2$-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 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, $I_1$ IBS antagonists block the cardiovascular effects of $I_1$ IBS agonists more effectively than pure $\alpha_2$-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 $\alpha_2$-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 $[^3H]$BU224 and $[^3H]$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.

$[^3H]$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 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 [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 α₂-adrenoceptors and IBS (Parker et al., 2004). Although agmatine is widely distributed throughout the body, binds to α₂-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<sub>A</sub> receptor agonist that also exhibits a low efficacy, partial agonist effect at GABA<sub>C</sub> 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<sub>1</sub> IBS in adrenal tissue and promote the release of arachidonic acid from PC12 cells (Prell et al., 2004). However, as a potential I<sub>1</sub> 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<sub>3</sub>R 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<sub>3</sub>R-mediated response (Chan et al., 1997; Eglen et al., 1998; Morgan et al., 1999). I<sub>3</sub> IBS agonists also interact with K<sup>+</sup>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<sub>3</sub>R agonists. Interestingly, IAA-RP was more potent than the prototypical I<sub>3</sub> agonist /I<sub>1</sub> antagonist efaroxan EC<sub>50</sub> =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 I₃ 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 Ca²⁺-dependent release from P₂ 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 I₃ 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 α₂-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 I₁ IBS agonists. These results would suggest that IBS ligands can modulate the activity of LC noradrenergic neurones through the activation of I₁ 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 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-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-HT₂C receptors and no affinity for 5-HT₁A 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 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) 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 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 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 rilmenidine 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 efaroxan, 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 (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 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 ($B_{\text{max}}$) 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 $[^{125}]p$-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 Gaₘ₁₁, Ga₁₂ 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 [³²H] 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-α₂
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.
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 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 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

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<table>
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<td>Harmane</td>
<td>Endogenous</td>
<td>↑ noradrenergic firing, ↓ serotoninerigic firing, ↑ serotonin, ↑ serotonin &amp; dopamine spontaneous neuronal activity</td>
<td>Locus coeruleus, Dorsal raphe, Hippocampus, Nucleus accumbans, Nucleus accumbans</td>
<td>Ruiz-Durantez et al., 2001; Touki et al., 2005; Ugedo et al., 1998; Adell et al., 1996; Baum et al., 1996; Ergene and Schoener 1993</td>
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<td>BU224</td>
<td>I&lt;sub&gt;2&lt;/sub&gt; ligand</td>
<td>↑ noradrenaline, ↑ noradrenaline</td>
<td>PVN, Frontal Cortex and hippocampus, Frontal Cortex, Hippocampus and hypothalamus</td>
<td>Finn et al., 2002; Hudson et al., 1999; Nutt et al., 1997; Finn et al., 2003; Finn et al., 2003</td>
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<td>2-BFI</td>
<td>I&lt;sub&gt;2&lt;/sub&gt; ligand</td>
<td>↑ noradrenaline, ↑ noradrenergic firing, ↑ noradrenaline</td>
<td>Frontal cortex and hippocampus, Locus coeruleus, Striatum</td>
<td>Nutt et al., 1995; Ugedo et al., 1998; Sastre-Coll et al., 2001</td>
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<td>BU98008</td>
<td>I&lt;sub&gt;2&lt;/sub&gt; ligand</td>
<td>↑ noradrenaline &amp; dopamine overflow</td>
<td>Frontal Cortex</td>
<td>Abu Ghazaleh et al., 2007</td>
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<td>Clonidine</td>
<td>I&lt;sub&gt;1&lt;/sub&gt;/I&lt;sub&gt;2&lt;/sub&gt; ligand</td>
<td>↑ noradrenergic firing, ↓ DOPA, 5-HTP synthesis</td>
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<td>Moxonidine</td>
<td>I&lt;sub&gt;1&lt;/sub&gt; agonist</td>
<td>↓ DOPA, 5-HTP synthesis</td>
<td>Cerebral cortex, Hippocampus</td>
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<td>Rilmenidine</td>
<td>I&lt;sub&gt;1&lt;/sub&gt; agonist</td>
<td>↑ neuronal activation, ↓ DOPA synthesis, ↓ 5-HTP synthesis</td>
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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 harmine, together with supporting evidence from (Diaz et al., 1997; Sanchez-Blazquez et al., 2000), suggest that these ligands may be agonistic in nature.
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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}\text{H}]\text{BU224}\ (I_2\ \text{selective ligand}), \[^{3}\text{H}]2\text{BFI}\ (I_2\ \text{selective ligand})\) and \[^{3}\text{H}]\text{rilmenidine}\ (I_1\ \text{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}\text{H}]\text{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 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 ± SEM (n = 5–7). **p < 0.01 and ++p < 0.01 compared with saline-treated controls at 30 and 60 min, respectively. ‡p < 0.05 and †p < 0.01 compared with BU224-treated controls at 30 and 60 min, respectively. SPSp < 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) 

- decreased DOPA & 5-HTP synthesis, increased NA & DA overflow/release, decreased NA turnover
- increased NA release, decreased DOPA & 5-HTP synthesis, decreased 5-HT turnover

- BU224: $K_i_{BU224} I_2 = 2.1 \text{nM}$, $K_i_{BU224} I_2 = 1.7 \text{mM}$
- 2-BFI: $K_i_{2-BFI} I_2 = 1.7 \text{mM}$

- Rilmenidine: $K_i_{Rilmenidine} I_1 = 7.1 \text{mM}$

(B) 

- Increased 5-HT release
- Increased 5-HTergic firing following systemic administration

- Harmamine binding

- Harmamine: $IC_{50} I_1 = 30.1 \text{nM}$, $IC_{50} I_2 = 49.4 \text{mM}$

- NAergic firing following ICV administration
(a) Noradrenaline (% Basal)

- Con + Sal (n = 5)
- Con + BU224 (10 mg kg\(^{-1}\)) (n = 5)
- AA + Sal (n = 5)
- AA + BU224 (10 mg kg\(^{-1}\)) (n = 5)

10 mg kg\(^{-1}\) BU224/saline i.p.

(b) Plasma Corticosterone (ng ml\(^{-1}\))

- Con + Sal (n = 6)
- Con + BU224 (10 mg kg\(^{-1}\)) (n = 6)
- AA + Sal (n = 8)
- AA + BU224 (10 mg kg\(^{-1}\)) (n = 6)

10 mg kg\(^{-1}\) BU224/saline i.p.