

Borne Identity: Leading Endogenous Suspects at Imidazoline Binding Sites

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Abstract

Over the past few years, a vast amount of research has shed light on the pharmacology of imidazoline binding sites (I-BS). To date, at least three classes of imidazoline binding sites have been characterised in accordance to their localisation, drug selectivity, proposed signalling pathways and functional roles. The existence of these sites raises the question as to whether an endogenous modulator exists. The identification of an endogenous extract denoted as clonidine displacing substance prompted the search for the active ingredient capable of mimicking the action of selective ligands at these sites. A number of candidates have been isolated and their functional activities have been assessed at these sites. Such endogenous ligands include agmatine, imidazoleacetic acid ribotide and the β -carboline harmaline. As of yet, no consensus has been made to confirm the identity of the endogenous ligand at I-BS. The current review collates and reports what is known about these substances and their functional significance at I-BS.

Keywords: Harmaline, Agmatine, β -carboline, CDS, Imidazoleacetic acid ribotide, Imidazoline binding sites

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The Heterogeneity of Imidazoline Binding Sites

The imidazoline binding sites (I-BS) represent a heterogeneous family of receptors/sites that stemmed from studies investigating the hypotensive effect of the imidazoline compound clonidine [1]. Structure-affinity relationship studies complemented by functional analyses characterised three types of I-BS, denoted I₁-BS, I₂-BS (I_{2A} and I_{2B}) and I₃-BS [2]. Extensive research has established the involvement of central I₁-BS in cardiovascular function [3]. Other reported functions include alleviating symptoms associated with metabolic syndrome X [4] and Huntington's Disease [5], promoting natriuresis [6], regulating intraocular pressure [7], and modulating mRNA expression for phenylethanolamine *N*-methyl transferase (PNMT) [8]. Furthermore, the expression of these sites was shown to be altered in conditions such as depression [9] and dysphoric premenstrual syndrome [10] suggesting their involvement in psychiatric conditions and endocrine function respectively. The current literature advocates the role of I₂-BS in a number of mood conditions, including depression [11] and anxiety [12]. Furthermore, a strong body of evidence has

demonstrated that I₂-BS ligands modulate neuropathic pain and analgesia [13-15]. These sites have also been implicated to play a role in attenuating symptoms associated with drug addiction [16], thermoregulation [17], acting as biomarkers for various neurodegenerative diseases and glial tumours [18] and appetite regulation [19]. Lastly, upon activation I₃-BS have been shown to exhibit insulinotropic activity as well as inhibit glucagon secretion from pancreatic cells implicating their potential importance in the management of type II diabetes [20].

The anatomical structures of I-BS remain inconclusive, as research has yet to verify the molecular nature of their binding proteins. A protein termed imidazoline receptor antisera selected (IRAS) was reported to represent the I₁-BS protein [21,22]. Later, a murine homologue of IRAS Nischarin was cloned [23] illustrating comparable results in biochemical and functional studies that are representative of I₁-BS [24-26]. The mitochondrial enzyme monoamine oxidase (MAO) is the most notably sought protein candidate for I₂-BS [27,28]. However,

various reports have illustrated that only a subset of I₂-BS reside on MAO, whilst a fraction of these sites were also found to be associated with brain creatine kinase [29]. Lastly, I₃-BS have been proposed to associate with the ion-conducting component Kir 6.2 coupled to K_{ATP} channels [30,31].

Proposed endogenous ligands at imidazoline binding sites

The existence of novel sites sensitive to imidazoline-related molecules raises the question as to whether an endogenous modulator does exist. Several candidates have been put forth based on their endogenous occurrence in man, affinity for these sites and their biological activity exerted *via* I-BS. The leading endogenous candidates include agmatine, imidazoleacetic acid ribotide and harmane (Figure 1).

The endogenous extract at imidazoline binding sites: clonidine displacing substance

In 1984, Atlas and Burstein isolated and partially purified an endogenous extract from rat and bovine brain homogenates. This extract was termed *Clonidine Displacing Substance* (CDS) due to its ability to displace specific [3H] clonidine binding in rat brain membrane [32]. CDS was reported to be most abundant in bovine lung [33]. Initially, CDS was proposed to be an endogenous modulator for α_2 -adrenoceptor (α_2 -AR) due to its affinity for these sites and its ability to mimic the actions of clonidine: brain CDS extract was shown to promote platelet aggregation and inhibit contractile response in rat vas deferens [34,35]. Conversely, the contractile response induced by serum CDS extracts in aortic tissue preparations was not blocked by the selective α_2 -AR antagonist rauwolscine [36]; illustrating that CDS does not exert its effect *via* α_2 -AR. Interestingly, whilst some groups reported that CDS elicits opposing effects to clonidine, others demonstrated the lack of clonidine-like properties of CDS [37,38]. The reported differences in CDS-evoked responses may be due to the variation in extraction techniques used to isolate and purify CDS, as well as the use of various tissue sources that has led to the ambiguity of its definitive role. CDS extracts from different tissue sources may not contain the same composition of active constituents that are present elsewhere which may account for differences in CDS activity. This notion was addressed by Pinthong et al. demonstrating that CDS extract derived from bovine brain and lung differ in activity; brain CDS attenuated the forskolin-stimulated cyclic adenosine monophosphate (cAMP) accumulation response

in guinea pig cerebral cortical preparations whilst lung CDS extract caused an elevation in response [39]. The latter effect was proposed to be mediated by histamine present in lung CDS extract [39]. The chemical composition of CDS is still a matter of debate due to the discrepancies in extraction procedures and the sources of CDS extracts. However, what is currently known about the chemical entity of CDS is that it does not resemble that of a catecholamine nor a peptide [40]. Various groups have identified a number of contaminants present in CDS extracts that have survived the extraction process, including biogenic amines and complex β -carbolines [41,42].

Binding analyses have shown that CDS displays affinity at I₁-BS in the rostral ventrolateral medulla (RVLM) and adrenal chromaffin cells [43], and at I₂-BS in rat brain membranes [44]. In cardiovascular studies, microinjection of CDS into rat RVLM potentiated a decrease in arterial blood pressure [45] similar to clonidine [1]. However, whether the hypotensive effect of clonidine is mediated solely by α_2 -AR or I₁-BS alone is still a matter of controversy. In contrast, Atlas' group reported an elevation in mean blood pressure following CDS treatment in cats [46] and rabbits [47]. The hypotensive effect elicited by clonidine was antagonised by CDS [46], suggesting that the component(s) in CDS extract interact at the same receptor as clonidine. In 1997, Chan and her colleagues demonstrated that rat brain-derived CDS extract stimulated glucose-dependent insulin secretion in rat and human isolated islets of Langerhans in a similar manner to efaroxan through its proposed interaction at atypical I₃-BS [48]. More importantly this was the first study to show that the imidazoline compounds, RX801080 and KU14R (I₃-BS antagonists), could block the secretagogue action of CDS [48]. At present, there has been no evidence to report the action of CDS extract on I₂-BS mediated effects. The endogenous expression of component(s) found in the CDS extract displaying affinity and functional activity at I-BS implies that a neuromodulator potentially exists for these sites, and various active components of CDS have been identified and were shown to elicit actions attributable to I-BS involvement.

Agmatine - the endogenous ligand at imidazoline binding sites?

Following the identification of an extract that was shown to display affinity and activity at I-BS it was crucial to isolate the principle component(s) associated with its pharmacology at these sites. In 1994, Li et al. extracted the decarboxylated product of L-arginine, namely agmatine, using ion and molecular weight exclusion chromatography followed by HPLC and mass spectrometry [49]. The presence of agmatine in purified extracts of CDS was shown to display affinity at α_2 -AR and I-BS, albeit weakly at the latter sites (K_i at I₁-BS=30 mM, K_i at I₂-BS=100 mM) [50]. Agmatine is synthesised enzymatically from arginine by membrane-bound mitochondrial arginine decarboxylase and is metabolised by agmatinase and/or diamineoxidase [38]. In neuronal preparations, agmatine was shown to exhibit neurotransmitter-like properties including vesicular storage and extrasynaptic release [51,52]. Furthermore, the cellular uptake of agmatine into mammalian cells was shown to be driven by the organic cation transporter 2 [53]. Thus, it is evident that agmatine fulfils a number of criteria to classify it as a neurotransmitter.

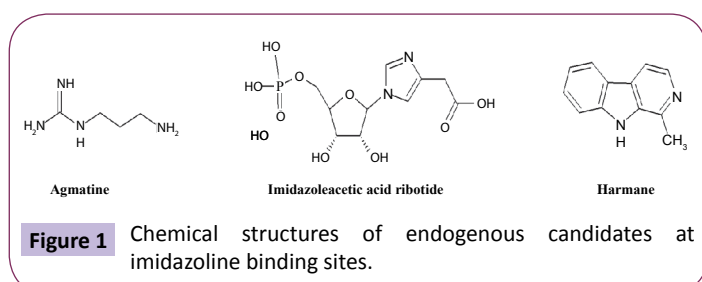


Figure 1 Chemical structures of endogenous candidates at imidazoline binding sites.

The pharmacological actions of agmatine have been thoroughly documented and shown to mimic some but not all of the properties of CDS. For instance, Regunathan et al. showed that agmatine induced the release of catecholamine from bovine chromaffin cells similarly to CDS [43]. In contrast, cardiovascular studies demonstrated that central administration of agmatine into the RVLM did not reproduce the central actions of clonidine on arterial blood pressure [54]. In behavioural studies, agmatine was shown to promote anticomulsive-like effects similarly to I₁-BS and I₂-BS selective ligands and was blocked by their respective I-BS antagonists [55]. Moreover, intracerebroventricular administration of agmatine in mice and rats produced an elevation in morphine, ethanol and nicotine induced antinociception; which was also observed with agmatine and the selective I₂-BS agonists 2-BFI [15,56,57] and more notably CR4056, which is currently underway for Phase II clinical trials [15]. Furthermore, the antinociceptive effect of agmatine was reversed by idazoxan and BU224; illustrating I₂-BS antagonism [56]. The exact method by which I₂-BS selective compounds potentiate opioid analgesia is yet uncertain. Studies have shown that I₂-BS ligands partly exert their effects through modulation of MAO activity [58]. In 1976, Iwamoto et al. demonstrated that acute treatment of mice with pargyline (irreversible non-selective MAO inhibitor) induced morphine antinociception in the tail-flick test; suggesting MAO involvement [59]. Moreover, morphine along with idazoxan (the first prototypical drug used to assess I₂-BS pharmacology) was shown to inhibit MAO_B activity in rat brain [60]; which further infers the role of MAO in morphine analgesia. However, it is unlikely that agmatine exerts its analgesic effect through MAO inhibition as agmatine did not affect MAO_B activity in rat brain homogenates [60]. As with the case of I₂-BS agonists, agmatine would appear to exert its analgesic response potentially through association with a subclass of I₂-BS that are not present on MAO, of which remains to be explored.

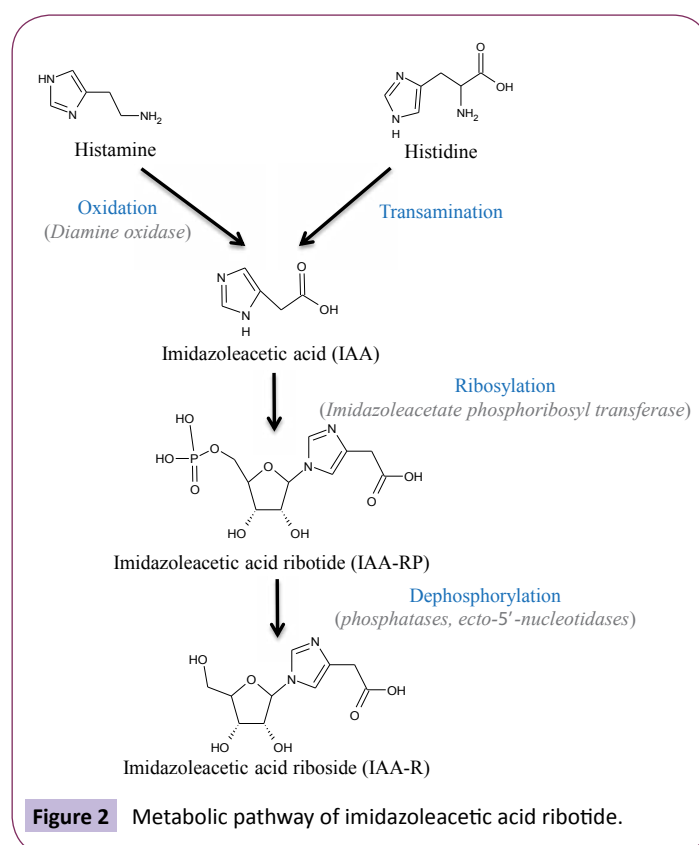
The current literature proposes that I₁-BS and I₂-BS play a role in major depression [61]. The expression of I₁-BS and I₂-BS were reported to be altered in platelets and brains of depressed patients [62-65]. In addition, the immunoreactivity of the I₁-BS protein candidate IRAS, as well as the proposed 29/30 kD I₂-BS protein, were also notably changed in platelets and brains of depressed suicide victims in comparison to healthy subjects [66,67]. In preclinical studies, selective I₂-BS compounds such as 2-BFI and BU224 exhibited antidepressant activity by regulating central monoamine levels [68,69] and increasing mobility time in the Porsolt forced swim paradigm [11,70,71]. Behaviour analyses showed that agmatine along with I-BS ligands (2-BFI, moxonidine and clonidine) mediated the antidepressant like effects of selective serotonin reuptake inhibitors (SSRIs). Furthermore, mice pretreated with SSRIs and exposed to the forced swim test showed overall elevated brain agmatine levels [72]. However, pretreatment of mice with an I-BS antagonist did not block the increase in brain agmatine concentration [72]. Nevertheless, the reported findings show that there is an apparent role of agmatine in mood conditions, which may in part be mediated by I-BS. Agmatine has also been shown to decrease plasma glucose levels in streptozotocin-induced diabetic rats and its lowering actions was hindered following pretreatment using the I₂-BS selective ligand BU224 [73]. The insulinotropic activity of agmatine was demonstrated

to be less efficacious than the I₃-BS ligand efaroxan [74]. Agmatine was shown to block K_{ATP} channels in isolated pancreatic β- cells [75] similarly to that illustrated by I₃-BS ligands [76].

All in all, does agmatine represent the bioactive component of CDS? In view of the current literature it appears that agmatine and CDS are two separate entities. The physicochemical properties of agmatine differ from that of CDS and the distribution of agmatine does not overlap with that of CDS [38]. Nonetheless, the emergent publications have proposed that agmatine does exert some of its effects through I-BS. However, its low affinity along with its weak activity at I-BS, in comparison to selective I-BS compounds, raises doubt on its fidelity for these sites. Agmatine has been reported to interact with other target sites including N-methyl-D-aspartate (NMDA) receptor [77], 5-HT₃ receptor channel [78], nicotinic receptor [79], which in turn may explain its eclectic biological effects.

Imidazoleacetic acid ribotide - The endogenous ligand at imidazoline binding sites?

Another proposed candidate is the phosphoribosylated derivative of imidazoleacetic acid (IAA). IAA is naturally occurring in brain, cerebrospinal fluid and plasma and has been suggested to play a role in brain function [80]. Histamine and histidine were reported to act as precursors for the enzymatic formation of IAA, with the latter being the most predominant pathway [80] (Figure 2). Early research by Ernsberger's group (1992) demonstrated that IAA displaced [³H] p-aminoclonidine binding from I₁-BS at micromolar



concentrations [81]. IAA administration into the lateral ventricle of the brain in cats elicited a hypotensive response [82]; corresponding to an I₁-BS mediated function [3]. The conjugate metabolite of IAA, namely IAA - ribotide (IAA-RP), was also reported to be present endogenously in rat tissue extracts and in human cerebrospinal fluid [83]. Immunostaining techniques detected the presence of IAA-RP in rat RVLM [83]; a region rich in I₁-BS [84]. In synaptosomal preparations, IAA-RP release was elicited by depolarizing concentrations of K⁺ and its release was noted to be Ca²⁺-dependent, a feature shared with numerous neurotransmitters [83]. Functional studies demonstrated that IAA-RP promoted arachidonic acid release from PC12 cells [83], a component associated in the signalling pathway for I₁-BS [85]. In rat brain, IAA-RP was reported to inhibit excitatory synaptic transmission [86,87]. Treatment with I₁-BS antagonist was shown to reverse the synaptic depression induced by IAA-RP, whilst rauwolscine (α₂-AR antagonist) was ineffective [86]. These studies infer that IAA-RP regulates brain synaptic currents through I₁-BS involvement but not α₂-AR. Further evidence to support the interaction of IAA-RP with I-BS showed that IAA-RP stimulated insulin secretion, which corresponds to I₃-BS function, and interestingly IAA-RP elicited a hypertensive response post microinjection in rat RVLM [83]. If IAA-RP were to represent the endogenous ligand for I-BS it would be assumed that it would act as an agonist at these sites. However, the activity of IAA-RP in cardiovascular studies does not correlate to the profile of I₁-BS agonists. In addition, the low binding affinity of IAA-RP at I₁-BS (K_i=13μM) in bovine adrenal medulla membranes [83] suggests that this compound is a weak endogenous candidate for I₁-BS. Moreover, there has been no report on the action of IAA-RP on I₂-BS - mediated functions and therefore the speculation of IAA-RP as an endogenous I-BS ligand remains inconclusive.

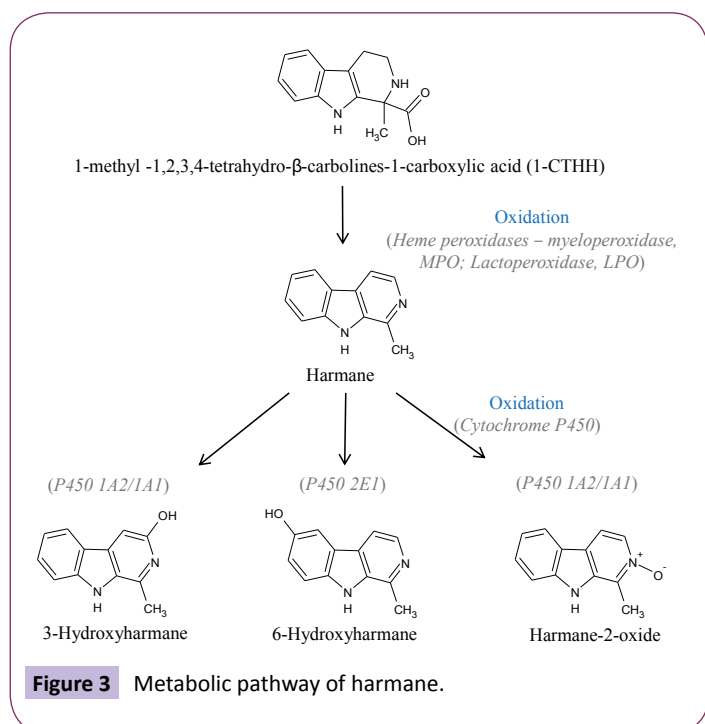
Harmane - the endogenous ligand at imidazoline binding sites?

One of the latest additions to the list of proposed endogenous molecules at I-BS is the β-carboline harmane [88]; which was reported to be the active constituent of CDS [41]. Harmane and other β-carbolines are naturally occurring in many plants and mammals [89]. In the mammalian body, β-carbolines are formed in a condensation reaction between indolealkylamines and aldehydes [90]. Recent advances have demonstrated that haem peroxidases catalyse the oxidation of (tetrahydro-β-carbolines) THβC to form harmane and norharmane [91]. Furthermore, these β-carbolines were shown to be metabolised by subtypes of cytochrome P450 enzymes [92] (**Figure 3**). Recent studies have shown that harmane accumulation in rat brain cortex does not represent an active-driven mechanism [93]. Furthermore, extracellular depolarizing concentrations of K⁺ failed to induce the release of [³H]harmane from brain cortical tissue; suggesting that harmane does not represent a classical neurotransmitter [93].

Harmane has been shown to interact with different classes of receptors including serotonin, dopamine, benzodiazepine, histamine and nicotine [94-96]. In addition, harmane has also been shown to interact with I-BS with a higher affinity (IC₅₀ at I₁-BS=31 nM, K_i at I₂-BS=49 nM) [97] compared to agmatine. The natural occurrence of harmane in the arcuate nucleus [98], a region rich in both I₂-BS [99] and MAOs [100], could suggest a possible role for harmane as an endogenous ligand at these sites.

Functional studies demonstrated that administration of harmane into rat brain RVLM elicited a hypotensive response which was also observed with I₁-BS ligands clonidine, rilmenidine, moxonidine and LNP 509 [101,102]. Furthermore, the central antihypertensive action of harmane and clonidine was reversed by the mixed α₂-AR/I₁-BS antagonist efaroxan. However, efaroxan was more efficacious against harmane than clonidine [101]. The hypotensive effect elicited by harmane is in agreement with the functional role of I₁-BS in the regulation of blood pressure at the level of the brainstem [3]. These findings along with the structure-affinity relationship of harmane binding to I₁-BS propose that harmane is a ligand at I₁-BS. In the rat central nervous system (CNS), harmane has been shown to modulate monoamine turnover [103] in a comparable manner to selective I₂-BS ligands such as 2-BFI and BU224 [68,70]. The regulation of central monoamine levels by harmane and I₂-BS compounds was proposed to occur partially through MAO inhibition [104]. Brain map imaging demonstrated that [³H]harmane distribution correlated with I₂-BS and MAO_A distribution [105,106], further strengthening the mode of action of harmane on these sites. In more recent studies, harmane was reported to elicit the spontaneous efflux of 5-HT from rat brain cortex devoid of MAO involvement [107]. Thus, these studies highlight that in addition to MOA inhibition harmane can partially regulate brain monoamine levels by inducing synaptic release.

A vast amount of research has explored the pharmacology of harmane at I-BS. Canular infusion of harmane into the dorsal hippocampus has been shown to stimulate feeding in rats [108] and this phenomenon was similarly documented with selective I₂-BS ligands [109]. Harmane has also been shown to induce



hypothermia in rats partially through MAO inhibition [110]. The homeostatic regulation of body temperature was also reported by high affinity ligands at I₂-BS namely 2-BFI, BU224 and CR4056 [17]; whilst no effect was observed by agmatine, another candidate endogenous ligand at I-BS [111]. Thus, it is likely that harmane potentiates its hypothermic response *via* I₂-BS association. In pancreatic β -cells, harmane induced insulin release through a proposed action at I₃-BS, a site known to regulate insulin secretion [20,112]. Although, ryanodine receptors and L-type calcium channels have also been suggested to play a role in mediating the insulinotropic activity of harmane [112]. The insulin secretagogue activity of harmane is analogous to that of CDS and efaroxan, such that they all antagonised the inhibitory response of diazoxide (K_{ATP} agonist) on glucose-mediated insulin release [113]. Furthermore, KU14R (I₃-BS antagonist) decreased the stimulatory effect of both harmane and efaroxan implicating that harmane mediates the secretion of insulin *via* a similar mechanism of action as efaroxan [113].

In behavioural studies, harmane has been shown to alleviate some symptoms associated with drug abuse and tolerance, which is thought to be mediated by I-BS [114]. Similarly, agmatine, BU224 and clonidine had also been reported to attenuate many behavioural characteristics of morphine abstinence syndrome [115]. In drug-discrimination models, harmane along with selective I₂-BS compounds were shown to potently and dose-dependently substitute for 2-BFI in the two-lever operant chamber model [116]. Moreover, harmane was also reported to substitute BU224 in drug discrimination studies [117]. The ability of harmane, along with I₂-BS agonists such as CR4056, to substitute for BU224 (I₂-BS antagonist) in discrimination studies is somewhat puzzling and requires further exploration. Previous approaches demonstrated that idazoxan also substituted for 2-BFI, but clonidine did not; illustrating specificity to I₂-BS interaction and not I₁-BS [118]. Interestingly, idazoxan displays antagonistic activity at I₂-BS. Therefore, it would have been expected that the antagonistic profile of idazoxan would attenuate rather than promote the discriminative stimulus effects. It was proposed that idazoxan

behaves as a partial agonist at I₂-BS in systems that require low efficacy demand, in the case of drug discrimination, and would act as a full antagonist in assays that require higher efficacy demand, such as pain modulation [119]. In more recent studies, Qiu et al. reported that I₂-BS ligands, including the proposed I-BS endogenous ligand harmane, substituted for CR4056 (high affinity I₂-BS ligand) in the two-lever drug-discrimination model [119]. Although harmane at 10mg/kg only displayed partial substitution, it was more potent than agmatine as it failed to substitute for CR4056 at the same dose [119].

Preclinical studies have shown that harmane exhibits anxiolytic and anti-depressant properties [120], which was also projected by I₂-BS ligands [11,70,121]. In restraint-stressed rats, harmane and BU224 treatment elevated Fos expression in distinct brain regions in a comparable manner; emphasizing the overlap of these molecules for the same target site [12]. Recently, Aglawe et al. described the anti-nociceptive activity of harmane to that of I₁-BS and I₂-BS agonists demonstrating similar functional responses [57]. However, whether the antinociceptive effect of harmane is preferentially elicited through its action on I₁-BS alone, I₂-BS alone or both needs to be addressed.

In light of the current literature it appears that harmane represents a strong candidate as an endogenous ligand at I-BS due to its high affinity for these sites and its ability to reproduce various effects that have been noted to occur by selective I-BS compounds. However, in order to truly crown harmane as an endogenous molecule its neurotransmitter properties need to be addressed to confirm its credibility to earn the title as a neurotransmitter/neuromodulator.

Conclusion

The emergent information on I-BS has shed light on their functional significance and importance in an array of medical conditions. The current review discusses the pharmacology of agmatine, imidazoleacetic acid ribotide and harmane as putative endogenous compounds at I-BS, reporting their functional overlap to known selective molecules for these sites. However,

the lack of a strong protein candidate for these sites has made it difficult to characterize the true identity of the endogenous ligand. Moreover, the discrepancies in the purification procedures and the controversial findings of some of the effects of these ligands need to be resolved in order to unravel the “borne identity” of the molecule for I-BS.

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