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Distribution of [^{3}H]BU224, a selective imidazoline I_2 binding site ligand, in rat brain

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Abstract

BU224 (2-(4,5-dihydroimidaz-2-yl)-quinoline) is a selective imidazoline I_2 binding site ligand characterised in both competition binding assays and functional studies. However, in some studies, BU224 has been reported to have a different functional effect from that seen with another selective imidazoline I_2 binding site ligand 2-BFI (2-(2-benzofuranyl)-2-imidazoline). This effect may reflect differing efficacies of the ligands or a difference in their brain distribution. The present study has investigated the distribution of the tritiated form of BU224 in rat brain and correlated this distribution with other imidazoline I_2 binding site ligands, [^{3}H]idazoxan and [^{3}H]2-BFI. Saturation studies revealed binding of [^{3}H]BU224 was of high affinity and saturable. The central distribution of [^{3}H]BU224 was similar to that previous reported for imidazoline I_2 binding site in rat brain. Autoradiography revealed that the highest levels of binding were in the arcuate nucleus, interpeduncular nucleus, area postrema, pineal gland and ependymal cell layer lining the ventricles. Correlation analysis of the binding distribution with our previous published studies revealed a highly significant correlation between [^{3}H]BU224 and both [^{3}H]idazoxan ($r = 0.94$) and [^{3}H]2-BFI ($r = 0.96$). These data indicate [^{3}H]BU224 labels the same population of imidazoline I_2 binding site in rat brain as seen with [^{3}H]idazoxan and [^{3}H]2-BFI. Therefore, the differences in functional effects observed with these compounds may reflect agonist and antagonist properties.

Keywords: Imidazoline binding site; Autoradiography; BU224 (2-(4,5-dihydroimidaz-2-yl)-quinoline; Brain, rat

1. Introduction

The family of imidazoline binding sites has been widely investigated using the imidazoline radioligands [^{3}H]clonidine, [^{3}H]*p*-aminoclonidine and [^{3}H]idazoxan. Based on their binding properties, biochemical and functional effects of a range of imidazolines and related compounds, this novel class of binding site has been subdivided into three distinct subtypes, the imidazoline I_1, imidazoline I_2 and imidazoline I_3 binding sites (for review, see Eglen et al., 1998). The imidazoline I_2 binding site has been further subdivided into the imidazoline I_{2A} and imidazoline I_{2B} subtypes based on the ability of amiloride to differentially displace [^{3}H]idazoxan from different tissue types and in different species (Parini et al., 1996). In the last few years, highly selective imidazoline I_2 binding site ligands have been developed including 2-BFI (2-(2-benzofuranyl)-2-imidazoline) and RS-45041-190 (4-chloro-2-(imidazolin-2-yl)-isoindolene) and characterised in biochemical and functional assays as well as in their radiolabeled forms (Milligan and MacKinnon, 1997).

Functionally, the imidazoline binding sites have been implicated in a number of disease states and may provide new therapeutic targets. The imidazoline I_1 binding site subtype is of particular interest in terms of control of blood pressure with drugs such as moxonidine and rilmenidine shown to reduce blood pressure (Bousquet et al., 1992). The imidazoline I_3 binding site subtype is an atypical imidazoline binding site, which may be associated with the K_{ATP}-channel and its location within the pancreas has highlighted it as a potential target for the treatment of diabetes (Chan, 1993). The imidazoline I_2 binding site subtype has been implicated in a number of diseases states, in particular, psychiatric disorders such as depression and opiate withdrawal (García-Sevilla et al., 1996; Sastre et al., 1996). Binding studies have demonstrated changes in the brain density of imidazoline binding site in postmortem

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tissue and evidence from neurochemical studies have further implicated the imidazoline I_2 binding site in a number of central nervous system (CNS) disease states, e.g. depression, Parkinson's disease and Huntington's Chorea (Hudson et al., 1999, 2001; Reynolds et al., 1996; Sastre et al., 1996; Ruiz et al., 1993; García-Sevilla et al., 1996).

BU224 (2-(4,5-dihydroimidaz-2-yl)-quinoline) is one of a series of quinoline derivatives shown to be selective for the imidazoline I_2 binding sites over α_2-adrenoceptors (Hudson et al., 1999). Radioligand binding studies in our laboratory revealed that BU224 has a high affinity for imidazoline I_2 binding sites with a K_i of 2.1 nM when displacing [^{3}H]2-BFI from rat brain membranes (Lione et al., 1998). Overall, the functional profile for BU224 is similar to that observed with other imidazoline I_2 binding site ligands, such as idazoxan and 2-BFI, but recent research in our laboratory and published in the literature suggests differences in the intrinsic activity of these compounds. Electrophysiological studies suggest that BU224 is an agonist at imidazoline I_2 binding sites in the dorsal horn of the rat spinal cord, stimulation of which induces antinociception (Diaz et al., 1997). Furthermore, this effect was blocked by idazoxan but only partially blocked by yohimbine supporting the involvement of the imidazoline I_2 binding sites. In contrast, Sanchez-Blazquez et al. (2000) showed an antagonist-like effect with BU224 against 2-BFI-induced potentiation of supraspinal morphine analgesia in mice. Studies in our own laboratory also suggest that BU224 and 2-BFI have different intrinsic activities at the imidazoline I_2 binding sites. In vivo brain dialysis studies have shown that BU224 but not 2-BFI elevates 5-hydroxytryptamine (5-HT) and dopamine levels in frontal cortex and striatum, respectively (Hudson et al., 1999). An alternative explanation for these results may be that these two selective imidazoline I_2 binding site ligands differ in the population of binding sites they label. In order to investigate the distribution of BU224 in the rat brain, the present study has used saturation binding studies and receptor autoradiography with [^{3}H]BU224 to determine the binding site population and distribution and compare this with our previous receptor autoradiography data for [^{3}H]idazoxan and [^{3}H]2-BFI (Lione et al., 1998).

2. Methods

2.1. Saturation binding studies

Saturation binding studies were performed using crude P_2 membranes prepared from male Wistar rats (250–300 g, Bantin and Kingman), killed by stunning followed by decapitation. The whole brain cellular membranes were isolated and binding studies were performed as described by Lione et al. (1998). Briefly, brains were homogenized and centrifuged initially in a Tris–sucrose buffer (0.32 M in 50 mM Tris–HCl, 1 mM Mg^{2+} pH 7.4 at 4 °C) then washed in assay buffer (50 mM Tris–HCl, 1 mM Mg^{2+} pH 7.4 at 4 °C). Membrane homogenate (400 μl, ~ 200 μg protein) was incubated to equilibrium (45 min) with 12 concentrations of [^{3}H]BU224 (0.01–10 nM) in triplicate with specific binding for each concentration defined using 10 μM 2-BFI in a final volume of 500 μl. Bound and free radioligand were separated by rapid filtration through presoaked (0.5% polyethyleneimine in distilled H_2O) Whatman GF/B filters using a Brandel M-24 cell harvester. Filters were then washed twice with 5 ml of ice-cold assay buffer and remaining membrane bound radioactivity was determined by liquid scintillation counting. Results were analysed using iterative nonlinear regression curve-fitting procedures (GraphPAD Prism, version 3.02). The protein content of the membrane preparations was measured using the methods described by Bradford (1976) and the results were expressed as femtomole bound per milligram protein.

2.2. Autoradiography

Preparation of sections for receptor autoradiography and binding were again performed according to the methods of Lione et al. (1998). Briefly, 12-μm sections were cut from phosphate-buffered saline-perfused male Wistar rat brains using a cryostat and mounted on gelatin-coated slides. Prior to binding, slides were washed for 20 min in assay buffer (50 mM Tris–HCl, 1 mM $MgCl_2$, pH 7.4, RT), then incubated for 30 min with 1 nM [^{3}H]BU224, ± 10 μM 2-BFI to determine the specific binding. The assay was terminated by two washes in ice-cold assay buffer. Sections were then dried and apposed to tritium sensitive film with tritium microscale standards (Amersham Life Science, UK) for 4–6 weeks photographic development. Quantitative analysis was performed using computer-assisted densitometry (MCID 5 analysis software supplied by Imaging

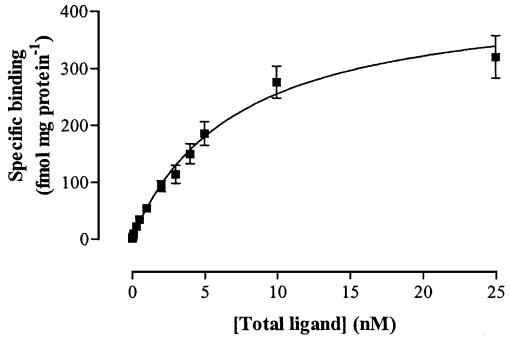

Fig. 1. Saturation curve for [^{3}H]BU224 binding to rat whole brain membranes. Specific binding was determined using 10 μM 2-BFI at each concentration and data analysed by iterative nonlinear regression and best fit to a single-site model. Results shown are mean ± s.e. mean (n = 8), calculated values for binding parameters were, K_D = 3.2 ± 1.7 nM and B_{MAX} = 119.5 ± 45 fmol mg protein^{-1}.

Research, UK) and the results were expressed as femtomole bound per milligram wet tissue equivalent. Data are shown as mean values for four animals from a single experiment. Brain regions were identified by reference to the atlas of Paxinos and Watson. Correlation analysis was performed using a two-tailed Spearman correlation test (Graphpad Prism, version 3.02).

2.3. Drugs and chemicals

[^{3}H]BU224 was custom-synthesized by NEN to a specific activity of 48 Ci mmol^{-1} and stored at −20 °C. The 2-BFI (2-(2-benzofuranyl)-2-imidazoline) was purchased from Tocris Cookson, UK. All chemicals used in the assay were of the highest grade and were purchased from Sigma Aldrich, UK.

3. Results

3.1. Saturation studies

The binding of [^{3}H]BU224 to rat whole brain membranes was saturable and of high affinity. The data were best fit to a single site-binding hyperbola, indicating that the [^{3}H]BU224 is binding to a single population of sites (Fig. 1). The calculated values for binding parameters were $K_D = 3.2 \pm 1.7$ nM and $B_{MAX} = 119.5 \pm 45$ fmol mg protein^{-1}, which are similar to those values observed for [^{3}H]2-BFI and [^{3}H]idazoxan (Lione et al., 1998).

3.2. Receptor autoradiography

The quantified results for [^{3}H]BU224 to rat brain sections are given in Table 1. The binding is highly localised with the highest levels of binding observed in regions close to the midline, particularly hypothalamic areas. This can be seen clearly in section F, Fig. 2, where high levels of binding can be seen in the arcuate nuclei, lateral mammillary bodies, the interpeduncular nucleus and pineal gland. Sections A–E in Fig. 2 show [^{3}H]BU224 binding to sections which again demonstrate the high levels of binding observed in these specific nuclei. In addition, a high level of binding is seen in the area postrema (section E, Fig. 2). Other brain areas showing medium levels of [^{3}H]BU224 binding were the paraventricular thalamic nucleus and medial habenular nucleus, the ventromedial hypothalamic nucleus, the dorsal raphe (section D, Fig. 2) and the ependymal layer lining the lateral ventricles. [^{3}H]BU224 binding to other brain areas quantified was <20 fmol mg wet tissue^{-1} and nonspecific binding was low <5 fmol mg wet tissue^{-1}, the limits of detection for quantification using the present methods.

Comparison of [^{3}H]BU224 with [^{3}H]2-BFI and [^{3}H]idazoxan suggests this novel radioligand binds to a similar population of binding sites with correlation coefficients of $r = 0.96$ and $r = 0.94$, respectively (Fig. 3). The correlation with [^{3}H]2-BFI and [^{3}H]idazoxan utilised data from our laboratory previously published by Lione et al. (1998) and correlated binding of each radioligand to the following brain areas: cerebellum C9, caudate putamen, frontal cortex, hippocampal fissure, nucleus solitary tract, medial and lateral habenular nucleus, dorsal raphe, substantia nigra,

Table 1
Quantified binding densities for different brain areas determined for [^{3}H]BU224 binding to rat brain sections

Regional distribution of [^{3}H]BU224 in rat brain sections			
No.	Abb	Brain area	Mean ± S.D. (fmol mg tissue^{-1})
1	AOM	anterior olfactory nucleus—medial	12.1 ± 3.1
2	AOV	anterior olfactory nucleus—ventral	10.9 ± 1.0
3	AOD	anterior olfactory nucleus—dorsal	9.70 ± 1.5
4	AOL	anterior olfactory nucleus—lateral	10.7 ± 1.7
5	aci	intrabulbar anterior commissure	4.31 ± 0.4
6	ox/sox	optic chiasm/supraoptic decuss	4.9 ± 0.6
7	Fr	frontal cortex	11.7 ± 1.0
8	Occ Ctx	occipital cortex	8.1 ± 1.0
9	Tem Ctx	temporal cortex	7.5 ± 1.0
10	Ent Ctx	entorrhinal cortex	10.9 ± 0.9
11	VO	ventral orbit	12.4 ± 0.9
12	cc	corpus callosum	4.9 ± 0.6
13	E	ependyma	27.3 ± 1.4
14	CPu	caudate putamen	10.6 ± 1.6
15	GP	globus pallidus	5.6 ± 1.0
16	Acb	nucleus accumbens	15.6 ± 2.5
17	CA1	hippocampal regions—CA1	10.5 ± 2.3
18	CA2	hippocampal regions—CA2	10.0 ± 2.7
19	CA3	hippocampal regions—CA3	10.1 ± 1.5
20	DG	dentate gyrus	12.5 ± 2.1
21	HiF	hippocampal fissure	16.5 ± 2.9
22	SuG	superficial grey layer, superior colliculus	12.6 ± 1.4
23	PVP	paraventricular thalamic nucleus	20.3 ± 2.6
24	MHb	medial habenular nucleus	24.0 ± 3.1
25	LHb	lateral habenular nucleus	8.72 ± 2.9
26	MD	mediodorsal thalamic nucleus	12.7 ± 4.0
27	DMD	dorsomedial	16.2 ± 2.6
28	VMH	ventromedial	21.6 ± 1.4
29	Arc	arcuate nucleus	98.5 ± 21.5
30	LM	lateral mammillary bodies	53.0 ± 13.9
31	BIC	nucleus brachium inferior colliculus	4.6 ± 1.1
32	IPN	interpeduncular nucleus	64.2 ± 16.3
33	SNR	substantia nigra	7.1 ± 4.8
34	VTA	ventral tegmental area	5.6 ± 2.9
35	CG	central grey	11.6 ± 1.4
36	DR	dorsal raphe	25.9 ± 3.0
37	Dtg	dorsal tegmental nucleus	6.3 ± 2.1
38	Pn	pontine nucleus	4.9 ± 1.9
39	AP	area postrema	91.6 ± 19.1
40	Sol	solitary tract	14.1 ± 4.5
41	Cere	cerebellum area 9	12.5 ± 2.5
42	Py	pyramidal tract	6.3 ± 2.0
43	Pi	pineal gland	38.4 ± 4.2

Results are shown as mean values ± S.D. (fmol mg^{-1} wet tissue equivalent) for each brain area measured in triplicate for four animals from a single experiment.

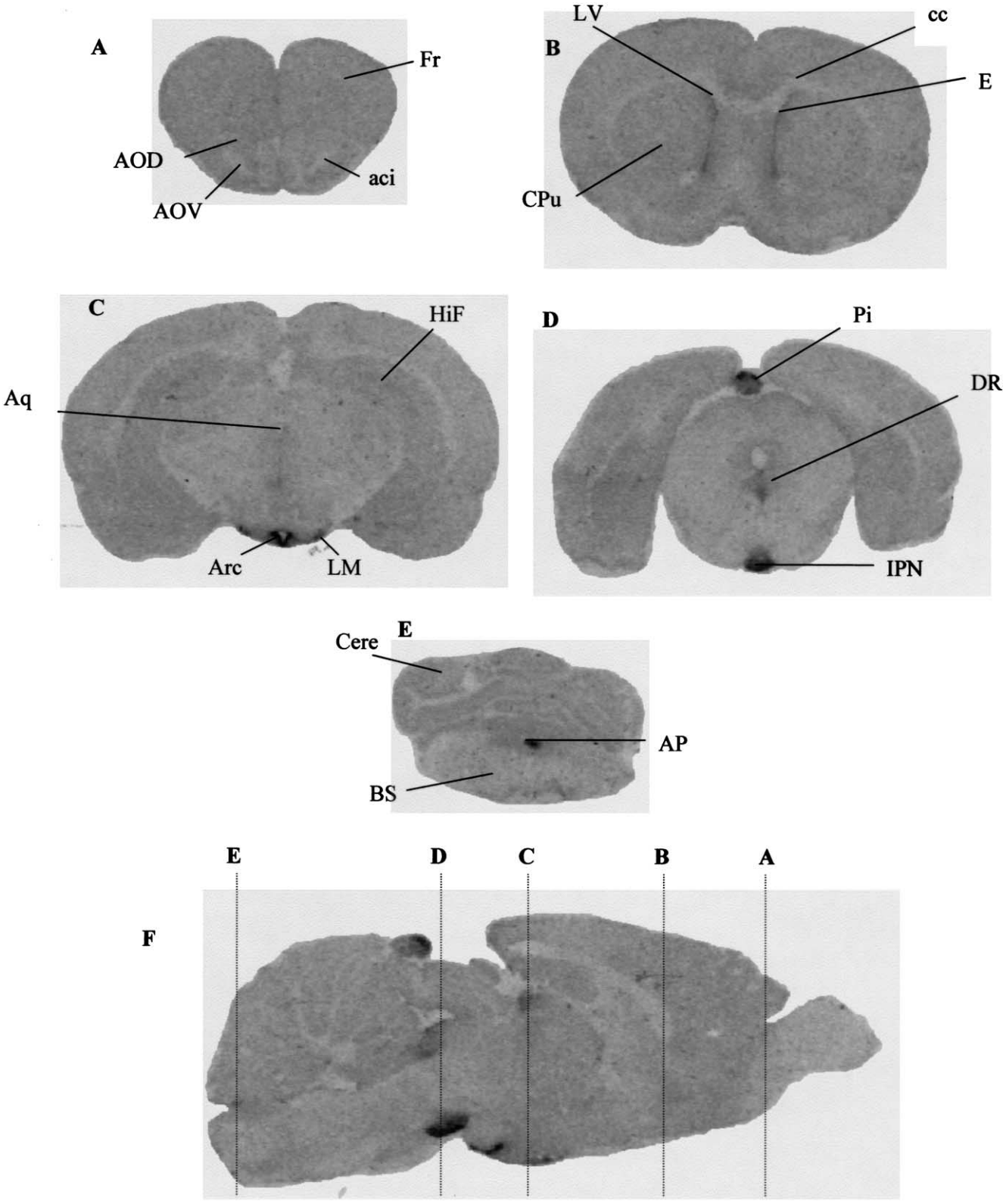

Fig. 2. Representative autoradiograms showing [^{3}H]BU224 binding to rat brain section. Sections taken at (A) bregma 4.2 mm; (B) bregma −0.26 mm; (C) bregma −3.3 mm; (D) −7.06 mm; (E) bregma −13.8 mm; (F) parasagital section approximately 0.4 mm from the midline. The locations of labels A–E correspond with transverse sections A–E.

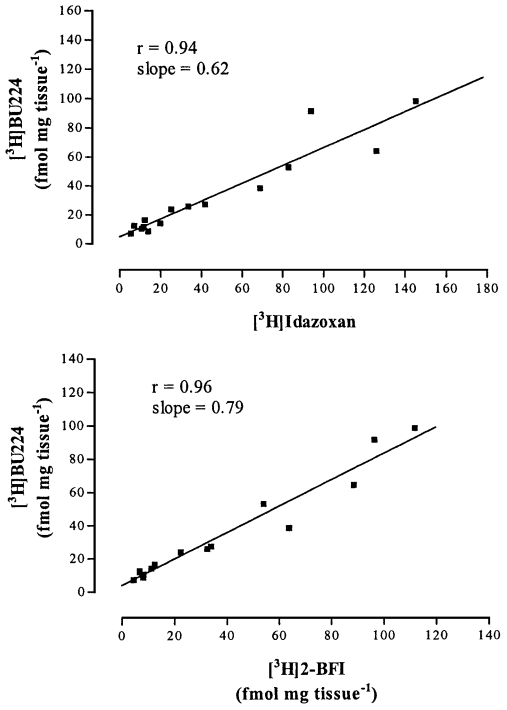

Fig. 3. Correlation analysis for [^{3}H]BU224 versus [^{3}H]idazoxan (top) and [^{3}H]2-BFI (bottom), data for comparison taken from Lione et al. (1998). Brain regions used for comparison were: cerebellum C9, caudate putamen, frontal cortex, hippocampal fissure, nucleus solitary tract, medial and lateral habenular nucleus, dorsal raphe, substantia nigra, ependyma, pineal gland, lateral mammillary bodies, area postrema, interpeduncular nucleus and arcuate nucleus.

ependyma, pineal gland, lateral mammillary bodies, area postrema, interpeduncular nucleus and arcuate nucleus.

4. Discussion

BU224 and 2-BFI are both highly selective imidazoline I_2 binding site ligands, although information on their intrinsic activity at this site is as yet unknown. Recently, studies have shown differences in the responses seen with these two ligands, suggesting that they may display differing efficacies at the imidazoline I_2 binding site. In particular, studies in our own laboratory have shown that BU224 elevates 5-HT and dopamine, whereas 2-BFI has little effect (Hudson et al., 1999). Interestingly, when monitoring the effects of these ligands on noradrenaline, a similar effect is observed with both ligands elevating noradrenaline in the frontal cortex and hippocampus. These differing neurochemical profiles are unlikely to be due to inhibition of monoamine oxidase directly as pretreatment with monoamine oxidase inhibitors have not been shown to attenuate the elevation in noradrenaline (Walford, personal communication).

Additional functional characterisation of BU224 and 2-BFI have shown both similarities and differences in their profiles. For example, in feeding studies, both BU224 (Edwards, personal communication) and 2-BFI (Jackson et al., 1995) increase food intake. However, a recent study investigating the effect of imidazoline I_2 binding site ligands on morphine-induced analgesia revealed differing activities of these ligands (Sanchez-Blazquez et al., 2000). 2-BFI was one of several imidazoline I_2 binding site ligands proposed to be an agonist in this model and the ability of 2-BFI to potentiate morphine-induced analgesia was reversed by both BU224 and idazoxan. Furthermore, IBI (isothiocyanatobenzyl imidazoline), an alkalating agent, which irreversibly binds to imidazoline I_2 binding sites, mimicked the effects seen with BU224 and idazoxan. In contrast, Diaz et al. (1997) proposed that BU224 was an agonist at the imidazoline I_2 binding sites based on its ability to induce spinal antinociception, although only α_2-adrenoceptor antagonists were investigated for their ability to block these effects and the authors did not discount an action by BU224 at α_2-adrenoceptors.

The explanations for these differing effects are complex and in vivo models are complicated by the possible presence of endogenous ligands. Moreover, the distribution of imidazoline I_2 binding site in the brain has only been modeled using either idazoxan or 2-BFI. Thus, one possible reason for the differing profiles of these two ligands may in fact relate to the binding site population in the brain. In order to investigate this, the distribution of imidazoline I_2 binding site labeled by [^{3}H]BU224 has been investigated with particular focus on regions in the brain associated with the different monoamines.

Characterisation of [^{3}H]BU224 in rat whole brain membranes showed that the affinity of BU224 was comparable to that seen with other radioligands for imidazoline I_2 binding site, namely [^{3}H]idazoxan and [^{3}H]2-BFI. [^{3}H]2-BFI and [^{3}H]idazoxan have been shown to label a single population of imidazoline I_2 binding site in rat brain homogenates with a K_D of 1.74 and 10.4 nM, respectively (Hudson et al., 1999). Similarly, binding of [^{3}H]BU224 was best fit to a single site model with a $K_D \sim 3.2$ nM and a total binding site population that was similar to that labeled by both [^{3}H]2-BFI and [^{3}H]idazoxan. The saturation binding data show that [^{3}H]BU224 has both a high affinity and specific binding to imidazoline I_2 binding sites, thus making a suitable ligand for receptor autoradiography.

The results presented here show that [^{3}H]BU224 binding is found throughout the brain with the highest levels seen within hypothalamic, mid and hind brain structures close to the midline. In particular, high levels of binding are seen in the arcuate nucleus and mammillary bodies of the hypothalamus, and both the interpeduncular nucleus and area postrema. In addition, high levels of binding are seen in the ependymal layer lining the ventricles. Binding to the dorsal raphe is also relatively high as well as the paraventricular thalamic nucleus and the medial habenular nucleus. Correlation with the distribution of imidazoline I_2 binding sites labeled by both [^{3}H]idazoxan and [^{3}H]2-BFI (data taken from Lione et al., 1998) suggest that all three radioligands label the same population of binding sites. More specifi-

cally, when looking at brain regions associated with the different monoamines, there does not appear to be any difference in the relative binding density. In particular, binding to the dorsal raphe, basal ganglia and substantia nigra are similar for each of the ligands. In context with previous functional studies, [^{3}H]BU224 does not appear to label a novel imidazoline I_2 binding site or a subpopulation of sites recognized by the other imidazoline I_2 binding site ligands. Therefore, the differing neurochemical profile and effect on nociception seen with both BU224 and 2-BFI is not due to the recognition of a different binding site population in the brain. Unfortunately, a lack of functional models for imidazoline I_2 binding site activation makes further characterisation of these compounds difficult. However, with the evidence presented in the present study, it is important that BU224 and 2-BFI, as well as the other imidazoline I_2 binding site ligands reported to have intrinsic activity, are tested both alone and in a challenge paradigm.

In conclusion, the similarity in the distribution of [^{3}H]BU224 with other known imidazoline I_2 binding site selective radioligands shows that this ligand labels the same population of binding sites. Therefore, it seems likely that differences in the functional profile of BU224 and 2-BFI relate to differences in their intrinsic activities at the imidazoline I_2 binding sites and may relate to agonist or antagonist actions.

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