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Imidazoline binding sites in Huntington's and Parkinson's disease putamen

Gavin P. Reynolds [a,*], Ruth M. Boulton [a], Sally J. Pearson [a], Alan L. Hudson [b], David J. Nutt [b]

[a] *Department of Biomedical Science, University of Sheffield, Sheffield S10 2TN, UK*
[b] *Psychopharmacology Unit, University of Bristol, University Walk, Bristol BS8 1TD, UK*

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Abstract

Binding of [^{3}H]2-(2-benzofuranyl)-2-imidazoline ([^{3}H]BFI) to the imidazoline I_2 receptor was determined in putamen taken post mortem from patients with two extrapyramidal motor disorders, Parkinson's and Huntington's diseases, and age-matched control subjects. No deficit of binding was apparent in Parkinson's disease, indicating that the receptors are not present on nigrostriatal terminals. A significant loss (by 56%) in imidazoline I_2 receptor binding was observed in Huntington's disease, consistent with the receptors being sited on degenerating neurons.

Keywords: Imidazoline I_2 receptor; Brain, human; Extrapyramidal disorder

The observation of non-adrenergic central effects of clonidine on blood pressure, and the identification of non-adrenergic imidazoline binding sites has resulted in a growing interest in functional roles of the imidazoline receptors. It is now established that there exist two types of these receptors: type 1, involved in blood pressure control, and type 2, of unknown function but with a relatively lower (μM) affinity for clonidine. Of particular interest is a possible link to motor function because of the relatively high density of imidazoline I_2 receptors in the basal ganglia (De Vos et al., 1991). For this reason we set out to study possible alterations in imidazoline I_2 receptors in the brains of patients with Parkinson's and Huntington's diseases.

The development of a specific radioligand [^{3}H]2-(2-benzofuranyl)-2-imidazoline (BFI) has facilitated the study of these sites. [^{3}H]BFI is a selective and specific imidazoline I_2 site ligand with low nanomolar affinity and little non-specific binding (Lione et al., 1995). We have used this ligand to determine I_2 sites in the putamen taken post mortem from patients with Parkinson's disease and Huntington's disease, and appropriate age-matched control subjects, using a modification of the method of Lione et al. (1995). Tissue was prepared by homogenization in 25 vols. of 50 mM Tris-HCl containing 1 mM $MgCl_2$ at pH 7.3, washed twice by centrifugation for 10 min at 34 000 $\times$ g at 4°C followed by rehomogenization. Incubations were carried out at 23°C for 45 min in 500 μl buffer containing [^{3}H]BFI and 200 vols. of tissue, following which the membranes were separated by filtration through Whatman GF/C paper and rapidly washed with ice-cold buffer. Bound radioligand was determined by scintillation counting. Due to the limited availability of both tissue and radioligand, experiments were undertaken with two concentrations of [^{3}H]BFI, 0.95 and 7.6 nM; the higher concentration provides a near-saturating level of binding to determine receptor sites, while the ratio of the lower to the higher concentrations is a function of receptor affinity and indicates whether differences in K_d may occur. Idazoxan at 10 μM was used to define specific binding. Statistical significances of differences between samples were determined by t-test.

The results are shown in Table 1. As there was no correlation with age in the two control series (Pearson

* Corresponding author. Tel.: (44) (114) 282-4662; fax: (44) (114) 276-5413.

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Table 1
Binding of [^{3}H]BFI to I_2 receptors in human putamen

	I_2 sites [a]	Binding ratio [b]
Control subjects ($n = 14$)	15.4± 5.4	0.36±0.08
Parkinson's disease ($n = 6$)	21.8±10.0 ($P = 0.05$)	0.31±0.05
Huntington's disease ($n = 8$)	6.8± 2.8 ($P < 0.001$)	0.49±0.07 ($P < 0.01$)

[a] Results are expressed as mean±S.D. of specific binding of 7.6 nM [^{3}H]BFI in pmol/g tissue. [b] The ratio of specific binding of [^{3}H]BFI at 0.95 nM to that at 7.6 nM. Statistical significances in comparison to control values are indicated in parentheses.

correlation coefficient = 0.06), these were pooled for comparison with the disease groups. The mean ratio of binding at the two concentrations varied between 0.31 and 0.49, equivalent to K_d values for the ligand of 3.5 to 1.4 nM and indicating that the higher concentration of ligand defined between 68% and 85% of total receptor density.

The absence of any deficit in the Parkinson's disease cases clearly indicates that imidazoline I_2 sites are not present on dopaminergic terminals in the human striatum, since a dopaminergic deficit was reflected by mean dopamine levels of 10.6% of control values in these tissues (results not shown). Interestingly, four of the Parkinson's disease cases exhibited binding of 7.6 nM [^{3}H]BFI to imidazoline I_2 sites that was above the control range, while one value, from a case dying of renal failure, was very low (5.8 pmol/g). Nevertheless, there was an overall significant increase in binding in the Parkinson's disease group, presumably reflecting an increase in receptor density; no significant difference in the binding ratio was apparent. This result is suggestive of an upregulation of imidazoline I_2 receptors in Parkinson's disease. It is unclear how this might be brought about although it could indicate an association of the endogenous ligand with one of the degenerating striatal inputs, that include serotoninergic neurons as well as the nigrostriatal pathway (Bernheimer et al., 1961).

There is a 56% deficit of binding to imidazoline I_2 sites in Huntington's disease, indicating that many of these receptors might be present on the neurons that degenerate in this disease. These include intrinsic striatal neurons containing γ-aminobutyric acid (GABA) and cholinergic interneurons, although glutamatergic input is also affected (Reynolds et al., 1990). Measurements of GABA and glutamate were performed on the samples studied here; the GABA deficits (57%) were similar to those of imidazoline I_2 sites. However, two control subjects did have imidazoline I_2 binding values typical of the Huntington's disease group (5.3 and 7.4 pmol/g) but with normal GABA concentrations above the range for Huntington's disease cases, which indicates that factors other than GABAergic neuronal density may determine the deficit in imidazoline I_2 receptors. In addition to the losses cholinergic neurons and glutamatergic terminals, one such factor may relate to cause of death, which might account for the single anomalous low result in the Parkinson's disease group discussed above. Nevertheless, although four Huntington's disease cases died of bronchopneumonia, this was not responsible for the reduction in imidazoline I_2 sites since one control and one Parkinson's disease case, each dying of bronchopneumonia, exhibited imidazoline I_2 binding values respectively within and above the control range. Several other neurotransmitter receptors have been reported to be affected by the striatal atrophy in Huntington's disease; these include the NMDA subtype of glutamate receptors (Dure et al., 1991), which has been proposed to mediate the neurotoxic process in Huntington's disease.

The results in Huntington's disease are not compatible with the suggestion that the imidazoline I_2 site is related to monoamine oxidase type B, perhaps by co-expression in glia (Sastre and Garcia-Sevilla, 1993), since this enzyme is substantially increased in the striatum in Huntington's disease (Mann et al., 1986).

The significant increase in binding ratio found in the Huntington's disease cases is likely to reflect an increase in radioligand affinity for the receptor. This could reflect loss of an endogenous ligand that normally exerts a competitive effect on radioligand binding at this site or, perhaps more likely, there may be differential loss of a subgroup of (lower affinity) receptors; certainly there are indications of imidazoline I_2 receptor heterogeneity in the human brain (Escriba et al., 1994). Further work will be required to examine this in more detail.

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