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Short communication

[^{3}H]Sumatriptan binding sites in human brain: regional-dependent labelling of 5-HT$_{1D}$ and 5-HT$_{1F}$ receptors

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Abstract

The general properties of [^{3}H]sumatriptan binding sites in postmortem human brain tissue sections are described. High concentrations of autoradiographic grains were seen in globus pallidus = substantia nigra > cortex > putamen > hippocampus. While 5-HT (5-hydroxytryptamine) displaced in all regions more than 90% of [^{3}H]sumatriptan binding, the level of binding inhibited by 5-CT (5-carboxamidotryptamine) varied in each region. Although the binding inhibited by 5-CT in some regions such as globus pallidus and substantia nigra was equivalent to that obtained with 5-HT, in cortical areas, such as frontal cortex and hippocampus, a substantial level of binding insensitive to 5-CT was seen. In addition, in membrane binding assays, 10 nM metergoline displaced most [^{3}H]sumatriptan specific binding in striatum and only 16% in frontal cortex. In the human brain sumatriptan binds to at least two 5-HT$_1$ receptors, 5-HT$_{1D}$ and 5-HT$_{1F}$.

Keywords: 5-Carboxamidotryptamine; 5-HT$_{1D}$ receptor; 5-HT$_{1F}$ receptor; Metergoline; Migraine; [^{3}H]Sumatriptan

1. Introduction

The application of molecular cloning techniques in the field of pharmacology has uncovered a diversity of neurotransmitter receptors. There is no better example of this multiplicity than 5-HT (5-hydroxytryptamine) receptors in general and 5-HT$_1$ receptors in particular, and to date six 5-HT$_1$-like receptors have been cloned: 5-HT$_{1A}$, 5-HT$_{1C}$, 5-HT$_{1D\alpha}$, 5-HT$_{1D\beta}$ (5-HT$_{1B}$), 5-HT$_{1E}$ and 5-HT$_{1F}$ (see, for review, Boess and Martin, 1994). The investigation of 5-HT$_{1A}$ receptors became possible when it was shown that 8-OH-DPAT (8-OH-2-di-*n*-propylaminotetralin), labelled with tritium, selectively bound to the 5-HT$_{1A}$ site. In the human brain 5-HT$_{1A}$ receptors are concentrated in the limbic system and raphe nuclei (Pazos et al., 1987). The 5-HT$_{1C}$ receptors, considered initially as 5-HT$_1$-like receptors on the basis of their high affinity for [^{3}H]5-HT, are present at high density in the choroid plexus (Pazos et al., 1984). As both the pharmacological profile and the nucleotide sequence of 5-HT$_{1C}$ receptors are closely related to those of 5-HT$_2$ receptors, 5-HT$_{1C}$ receptors are now classified as a subtype of 5-HT$_2$ receptors, the 5-HT$_{2C}$ receptor subtype (see, for review, Zifa and Fillion, 1992; Boess and Martin, 1994).

The 5-HT$_{1D}$ receptor subtype has been classically defined as the labelling of [^{3}H]5-HT in the presence of critical concentrations of 8-OH-DPAT and mesulergine. The regional distribution of 5-HT$_{1D}$ receptors in the mammalian brain is heterogeneous. In the substantia nigra, basal ganglia and striatonigral pathway these sites account for 90% of the 5-HT$_1$ binding (Waeber et al., 1990). 5-HT$_{1D}$ receptors also have been identified in porcine coronary artery and in canine basilar artery. The presence of these receptors in the vascular bed may be responsible for some pharmacological properties of the 5-HT$_{1D}$ receptor agonists. Sumatriptan, the new antimigraine drug, was presented as one of the most selective agonists for the 5-HT$_{1D}$ receptor (Peroutka, 1990). Two human 5-HT$_{1D}$ receptor subtypes, named 5-HT$_{1D\alpha}$ (Hamblin and Metcalf,

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1991; Weinshank et al., 1992) and 5-$HT_{1D\beta}$ (Demchyshyn et al., 1992; Weinshank et al., 1992), have recently been cloned. In addition, one further 5-HT_1 receptor subtype for which sumatriptan also shows nM affinity, 5-HT_{1F}, has been cloned recently and pharmacologically characterized (see, for review, Martin and Humphrey, 1994; Boess and Martin, 1994). Finally, 5-HT_{1E} receptors correspond to those binding sites displaying high affinity for [^{3}H]5-HT and low affinity for 5-CT (5-carboxamidotryptamine) and ergotamine. These receptors are concentrated in the cortex and striatum (Leonhardt et al., 1989). These data must be taken into account when analysing the affinity of serotonergic compounds for 5-HT_1 receptors.

In the present study we show the general properties of [^{3}H]sumatriptan binding sites in human brain tissue sections.

2. Materials and methods

2.1. Human brain tissues

Postmortem human brain tissues from 5 subjects (age = 47 ± 15 years; postmortem delay = 21 ± 8 h) who had died without a history of neuropsychiatric disease were obtained at necropsy. Immediately after autopsy, the brains were halved sagitally, one half being used in radioligand binding assays, and the other half being fixed in 10% formalin for neuropathological examination. Blocks of multiple brain areas were frozen on dry-ice and transferred to storage at −80°C.

2.2. Autoradiographic assays

For the autoradiographic assays, sections of the most representative brain areas (12 μm thick) were cut using a microtome cryostat. After preliminary assays, in which several conditions for incubation (30, 60 and 90 min) and washing (5 min, 2 × 5 min and 2 × 30 s) were analysed with 4, 7 and 10 nM [^{3}H]sumatriptan, sections – in duplicate – were incubated in a 0.17 M Tris-HCl buffer (pH = 7.4), containing 4 mM $CaCl_2$ and 0.01% of ascorbic acid, with 4 nM [^{3}H]sumatriptan (67 Ci/mmol, Amersham) and in the presence of 10 μM pargiline for 60 min at room temperature, after a 30 min preincubation in the same buffer. The slide-mounted sections were dipped and rinsed twice for 5 min in the same buffer, briefly dipped in cold water and then dried in a cold air stream. The non-specific binding was determined in two consecutive sections incubated in the presence of 10 μM 5-HT and 0.1 μM 5-CT, respectively. After 3 months exposure to tritium-sensitive films, together with the appropriate standards, the films were developed.

2.3. Membrane binding assays

Membranes from human frontal cortex and posterior striatum were prepared as already described (Pazos et al., 1984). Briefly, tissues were homogenized in 0.32 M sucrose and this homogenate was centrifuged at 70 000 × g for 15 min. The pellet was resuspended, incubated at 37°C for 15 min and centrifuged again. This final pellet was resuspended in 50 mM Tris-HCl buffer (pH 7.7) containing 4 mM $CaCl_2$ and 0.1% ascorbic acid and stored in liquid nitrogen until used.

The binding of [^{3}H]sumatriptan (0.5–22 nM) to these tissues was measured in triplicate at equilibrium in 1-ml aliquots of the membranes. The samples were incubated for 30 min at 37°C in the same buffer and also following the conditions already described (Pazos et al., 1984). The specific binding was defined with 10 μM 5-HT. The influence of 10 nM metergoline on specific binding of 5 nM [^{3}H]sumatriptan was tested in consecutive aliquots.

2.4. Data analysis

The autoradiograms were analysed and quantified (Unnerstall et al., 1982) using a computerized image analysis system (MICROM-IP, Microm España, Barcelona, Spain). Saturation membrane binding parameters were estimated by Scatchard analysis. Results are expressed as mean ± S.D.

3. Results

3.1. Autoradiographic assays

Variable numbers of specific [^{3}H]sumatriptan binding sites were observed throughout the human brain. The highest concentrations of autoradiographic grains were seen in globus pallidus and substantia nigra. Fronto-temporal cortex, caudate-putamen and hippocampus, in this order, also showed moderate levels of binding. Low densities were observed, in general terms, in regions such as cerebellum, pons, medulla oblongata and spinal cord. 5-HT consistently displaced in all regions more than 90% of [^{3}H]sumatriptan binding.

As is shown in Table 1 and Fig. 1, the binding inhibited by 0.1 μM 5-CT in some regions, such as globus pallidus and substantia nigra, was equivalent (more than 95%) to the total specific binding obtained with 5-HT. However, the percentage of specific binding insensitive to 5-CT in other areas such as the CA_1 field of the hippocampus (67.7%) or frontal cortex (43.9% in layers I–IV, 66.9% in layer V and 55.1% in layer VI) was clearly substantial.

Table 1
Density of [^{3}H]sumatriptan binding sites in human brain tissue sections

Brain area	Density fmol/mg tissue	% binding insensitive to 0.1 μM 5-CT
Frontal cortex		
layers I–IV	8.7±2.9	43.9
layer V	14.5±4.0	66.9
layer VI	12.7±6.6	55.1
Hippocampus, CA_1 field	4.1±0.8	67.7
Putamen	7.5±2.0	<5
Globus pallidus	19.2±2.0	<5
Substantia nigra	17.0±7.2	12

3.2. Membrane binding assays

The binding of [^{3}H]sumatriptan was specific, saturable (data not shown) and of high affinity (the calculated K_d value being between 10 and 15 nM). 10 nM metergoline was able to displace 97% of the [^{3}H]sumatriptan specific binding in the striatum and only 16% of the [^{3}H]sumatriptan specific binding in the frontal cortex.

4. Discussion

To our knowledge, this is the first report describing [^{3}H]sumatriptan binding sites in human brain tissue sections. Although a complete mapping study (in preparation) will have to provide more exact information about the quantitative distribution of this new radioligand, the anatomical distribution of [^{3}H]sumatriptan binding sites is comparable, in general terms, to that described for the 5-HT_{1D} receptor as labelled with its selective radioligand [^{125}I]GTI (serotonin-*O*-carboxymethylglycyl-tyrosinamide) (Bruinvels et al., 1994a). However, one interesting finding of this study is the fact that, unlike 5-HT, 5-CT and metergoline cannot displace a substantial part of [^{3}H]sumatriptan binding sensitive to 5-HT in cortical areas. Both 5-CT and metergoline are very potent 5-HT_{1D} receptor ago-

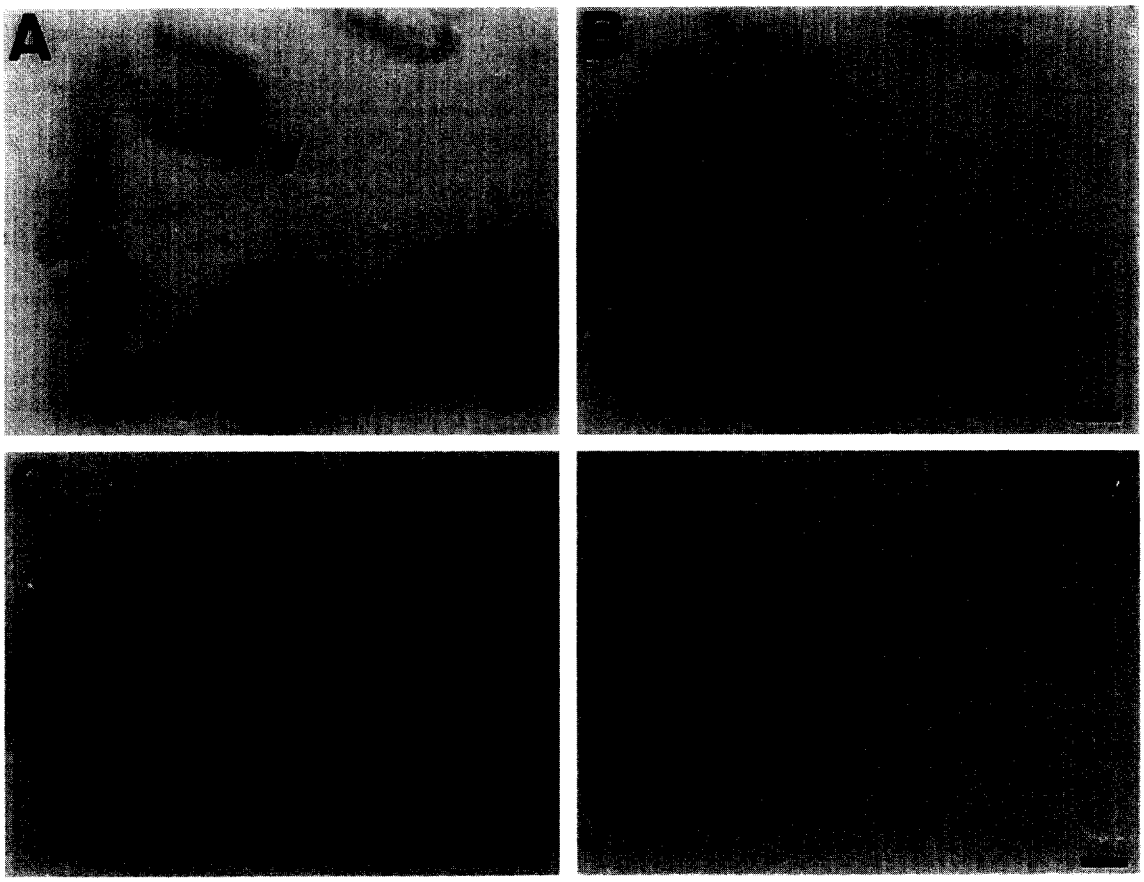

Fig. 1. Representative autoradiographic images of [^{3}H]sumatriptan binding to frontal cortex (A, B) and posterior striatum (C, D) sections. A, C: total binding. B, D: non-specific binding in the presence of 5-CT. Notice the high level of binding in globus pallidus and the moderate binding in putamen (image C), as well as the complete displacement of the total binding by 5-CT in posterior striatum (image D). Also notice the significant levels of [^{3}H]sumatriptan binding sites in frontal cortex (image A) and the incomplete displacement of the total binding with 5-CT (image B) when compared with 5-HT (not shown). GP = globus pallidus; P = putamen; I–IV = layers I–IV of the frontal cortex; V = layer V of the frontal cortex. Bars = 2 mm.

nists, their reported nM K_i for 5-HT_{1D} receptor ranging from 0.6 to 2.9 for 5-CT and from 0.8 to 7.8 for metergoline (see, for review, Boess and Martin, 1994; Zifa and Fillion, 1992). This means that sumatriptan binds, in the human brain, to at least one other 5-HT_1 receptor subtype. This receptor is neither the 5-HT_{1A} nor the 5-HT_{1E} receptor subtype because of the low affinity, in the μM range, that sumatriptan shows for these 5-HT_1 receptor subtypes (Zifa and Fillion, 1992; Leonhardt et al., 1989). There is some evidence suggesting that this receptor is the recently cloned 5-HT_{1F} receptor subtype. Firstly, this is the only 5-HT receptor subtype, leaving the 5-HT_{1D} receptor subtypes aside, for which sumatriptan shows high affinity, with a reported K_i value of 23 nM (Adham et al., 1993). Secondly, the expression of the 5-HT_{1F} receptor mRNA in the guinea-pig brain in significant amounts occurs only in some cortical regions, frontal cortex and hippocampus, being highest along layer V of the frontal cortex, which concurs with the distribution of [^{3}H]sumatriptan binding sites insensitive to 5-CT and metergoline found in human brain in our assays (Adham et al., 1993; Bruinvels et al., 1994b). In addition, very recently it has been shown that [^{3}H]sumatriptan labels putative 5-HT_{1F} receptor binding sites in the guinea-pig brain (Rhodes et al., 1995).

The potential implications of our findings warrant a final comment. After the discovery of sumatriptan it was clear that the receptor mediating its vascular effects was not the 5-HT_{1D} receptor subtype itself but an unknown '1D-like' receptor (Parsons, 1991). Although there is now reasonable evidence suggesting that sumatriptan produces vascular contraction via the 5-$HT_{1D\beta}$ receptor subtype (Hamel et al., 1993), it is possible that along with its effects on 5-HT_{1D} receptors, some of the actions of sumatriptan are mediated by at least one other 5-HT_1 receptor, the 5-HT_{1F} receptor subtype.

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