Agonist-dependent modulation of G-protein coupling and transduction of 5-HT$_{1A}$ receptors in rat dorsal raphe nucleus

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Abstract

5-HT$_{1A}$ receptors couple to different G$_o$/G$_i$ proteins in order to mediate a wide range of physiological actions. While activation of post-synaptic 5-HT$_{1A}$ receptors is mainly related to inhibition of adenyl cyclase activity, functionality of autoreceptors located in raphe nuclei has been classically ascribed to modifications of the activity of potassium and calcium channels. In order to evaluate the possible existence of agonist-directed trafficking for 5-HT$_{1A}$ autoreceptors in the rat dorsal raphe nucleus, we studied their activation by two agonists with a different profile of efficacy [[(+]8-OH-DPAT and buspirone], addressing simultaneously the identification of the specific G$_a$ subtypes ([³²S]GTPyS labelling and immunoprecipitation) involved and the subsequent changes in cAMP formation. A significant increase (32%, $p<0.05$) in (+)-8-OH-DPAT-induced [³²S]GTPyS labelling of immunoprecipitates was obtained with anti-G$_{ia1}$ antibodies but not with anti-G$_{ia2}$, anti-G$_{ia3}$, anti-G$_{ia4}$, anti-G$_{ia5}$ or anti-G$_{ia6}$ antibodies. In contrast, in the presence of buspirone, significant [³²S]GTPyS labelling of immunoprecipitates was obtained with anti-G$_{ia2}$ (50%, $p<0.01$), anti-G$_{ia3}$ (32%, $p<0.01$) and anti-G$_{ia4}$ (29%, $p<0.05$) antibodies, without any labelling with anti-G$_{ia5}$, anti-G$_{ia6}$ or anti-G$_{ia7}$. The selective 5-HT$_{1A}$ antagonist WAY 100635 blocked the labelling induced by both agonists. Furthermore, [(+]8-OH-DPAT failed to modify forskolin-stimulated cAMP accumulation, while buspirone induced a dose-dependent, WAY 100635-sensitive, inhibition of this response ($I_{max} 30.8\pm4.9$, $pEC_{50} 5.95\pm0.46$). These results demonstrate the existence of an agonist-dependency pattern of G-protein coupling and transduction for 5-HT$_{1A}$ autoreceptors in native brain tissue. These data also open new perspectives for the understanding of the differential profiles of agonist efficacy in pre- vs. post-synaptic 5-HT$_{1A}$ receptor-associated responses.

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Introduction

The neurotransmitter serotonin (5-HT) exerts its actions through the activation of at least 14 different receptor subtypes, from 5-HT$_1$ to 5-HT$_7$ (Hoyer et al. 2002). Substantial data exist to support a link between the serotonergic system and the physiopathology of neuropsychiatry diseases. In this regard, 5-HT$_{1A}$ receptors, present in high concentrations in the central nervous system, appear to be of special relevance: adaptive changes in the functionality of this subtype are believed to underline the therapeutic effectiveness of anxiolytic and antidepressant drugs (Blier & Ward, 2003; Hensler, 2003). 5-HT$_{1A}$ receptors are present in high densities in limbic brain areas (hippocampus, lateral septum), cortical areas (particularly prefrontal and entorhinal cortex), as well as in the raphe nuclei, both dorsal raphe nucleus (DRN) and median raphe nucleus (MRN) (Pazos & Palacios, 1985; Pompeiano et al. 1992). While those receptors in limbic and cortical areas are post-synaptically localized, 5-HT$_{1A}$ receptors in raphe nuclei are localized in cell bodies and dendrites, where they act as autoreceptors (Sotelo et al. 1990). 5-HT$_{1A}$ receptors are coupled to the G$_{i/o}$ family of G proteins, which include pertussis toxin-sensitive G$_{ia}$, G$_{ib}$, G$_{ic}$ and G$_{is}$ and pertussis toxin-insensitive G$_z$ proteins (reviewed in Raymond et al. 2002).
In heterologous recombinant systems, 5-HT\textsubscript{1A} receptors preferentially interact with \(G\textsubscript{ia}\) subunits, and, to a lesser extent, with other members of the group: \(G\textsubscript{ia}<G\textsubscript{oa}<G\textsubscript{ox}\) (Bertin et al. 1992; Fargin et al. 1991; Garnovskaya et al. 1997; Liu et al. 1994; Newman-Tancredi et al. 2002). Interaction of 5-HT\textsubscript{1A} receptors with \(G\textsubscript{ia}\) and \(G\textsubscript{oa}\) is very weak (Raymond et al. 1993). In rat brain tissue, a regional pattern of preferential coupling to \(G\) subunits has been reported for this receptor (Mannoury la Cour et al. 2006).

On the basis of agonist-directed trafficking of receptor signalling theory (Kenakin, 1995), it is now widely accepted that, depending on the nature of the agonist, receptor stimulation will selectively activate one specific \(G\)-protein subtype and downstream transduction pathway (Kenakin, 1995; Newman-Tancredi et al. 1997). Coupling of 5-HT\textsubscript{1A} receptors to \(G\textsubscript{ia}\) proteins leads to the mediation of a range of actions that include inhibition of adenylyl cyclase (AC), opening of \(K^+\) channels, closing of \(Ca^{2+}\) channels, and stimulation of phospholipase \(C\beta\) and mitogen-activated protein kinase (MAPK) (Raymond et al. 2001).

5-HT\textsubscript{1A} autoreceptors localized on raphe nuclei play a critical role in the regulation of the functionality of 5-HT neurotransmission: in the rat DRN, activation of 5-HT\textsubscript{1A} receptors results in the inhibition of neuronal cell firing (Sprouse & Aghajanian, 1987). Modulation of these autoreceptors is therefore of high relevance in the mechanism of action of 5-HT-acting drugs, including antidepressants and antipsychotics. Hyperpolarization through opening of potassium channels (Blier et al. 1993; Innis & Aghajanian, 1987; Penington & Kelly, 1990; Sprouse & Aghajanian, 1987), as well as inhibition of voltage-dependent calcium currents (Chen & Penington, 1996; Penington & Kelly, 1990) have been identified as post-receptor mechanisms linked to the activation of 5-HT\textsubscript{1A} receptors in rat DRN; however, no inhibition of AC activity has been reported following activation with agonists such as (+)-8-OH-DPAT (Clarke et al. 1996; Johnson et al. 1997) or 5-CT (Clarke et al. 1996). Interestingly, some experimental evidences for functional selectivity regarding 5-HT\textsubscript{1A} receptors have been recently published (Newman-Tancredi et al. 2009).

It is noteworthy that the pharmacological profile of different 5-HT\textsubscript{1A}-acting drugs depends on the synaptic localization of the receptor: while several compounds, i.e. (+)-8-OH-DPAT, show a high level of efficacy regardless the pre- or post-synaptic localization, behaving as full agonists, other drugs, such as buspirone, show a complex profile: low efficacy at post-synaptic receptors (partial agonist), but significantly higher efficacy at 5-HT\textsubscript{1A} receptors in raphe nuclei (Sussman, 1998). Although this difference has been classically explained in terms of spare receptors (Meller et al. 1990), the possibility exists that it is really reflecting a case of functional selectivity.

In order to evaluate the existence of agonist dependence in 5-HT\textsubscript{1A} receptor activation of specific subtypes of \(G\) proteins and downstream transduction pathway at the level of rat DRN, we have analysed the activation of this receptor by two agonists with different profiles of efficacy: (+)-8-OH-DPAT (full agonist) and buspirone (partial agonist), studying the subtype of \(G\alpha\) coupled (immunoprecipitation of \(^{[35S]}G\text{TP}_{\gamma}S\) labelled \(G\alpha\) subunits) and comparing it with their effects on cAMP inhibition.

### Material and methods

#### Animals

Male Wistar rats weighing 200–250 g were group-housed and maintained on 12 h light/dark cycle (lights on 08:00 hours), with access to food and water available \textit{ad libitum}. All experimental procedures were performed according to Spanish legislation and the European Communities Council Directive on ‘Protection of Animals Used in Experimental and Other Scientific Purposes’ (86/609/EEC) in accordance with the Declaration of Helsinki.

#### Reagents

\(^{[35S]}G\text{TP}_{\gamma}S\) (1250 Ci/mmol) was purchased from DuPont NEN (Brussels, Belgium). d.L-dithiothreitol (DTT), guanosine 5'-diphosphate sodium salt (GDP), guanosine 5'-triphosphate sodium salt hydrate (GTP), guanosine-5-O-(3-thio)triphosphate (GTP\textsubscript{S}), adenosine 5'-triphosphate disodium salt (ATP), ethylene-diaminetetraacetic acid (EDTA), ethylene glycol-bis (\(\beta\)-aminoethly ether)-\(N,N,N',N'\)-tetraacetic acid tetrasodium salt (EGTA), leupeptin, 3-isobutylmethylxanthine (IBMX), phosphocreatine, creatine phosphokinase myokinase, forskolin, Chaps, phenylmethylsulfonylfluoride; aprotinin, leupetin, pestatin, antipain, chomostatin, sodium deoxicholate, igepal, (+)-8-hydroxy-2-di-n-propylamino-tetralin ([(+)-8-OH-DPAT] and [N-[2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl]-N-2-pyrindyl-cyclohexanecarboxamide]-maleate (WAY 100635) were obtained from Sigma-Aldrich (USA). Buspirone were obtained from RBI (Spain). SB 269970 was from Tocris Cookson Ltd (UK). All other chemicals used were analytical grade. Rabbit anti-\(G\text{ia}\) (sc-391), anti-\(G\text{ia}\) (sc-7276), anti-\(G\text{ia}\) (sc-262), anti-\(G\text{ia}\) (sc-388) and anti-\(G\text{ia}\) (sc-387) were purchased...
from Santa Cruz Biotechnology Inc. (Germany), GB-Sepharose beads were from GE Healthcare Ltd (UK).

**Tissue preparation**

Rats were killed by decapitation. Their brains were quickly removed and the dorsal raphe area was carefully dissected at cold temperature (0–4 °C) by a series of steps as previously reported (Echizen & Freed, 1983) and stored at −80 °C until required. For autoradiography experiments, coronal sections of 20 μm thickness were cut at −20 °C using a microtome cryostat (Microm HM550, Germany) and thaw-mounted in gelatinized slides and stored at −20 °C until required.


Labelling of brain sections with[^14]S[^35]GTPγS was performed as described previously (Castro et al. 2003). Slide-mounted sections were preincubated for 30 min at room temperature in a buffer containing 50 mM Tris–HCl, 0.2 mM EGTA, 3 mM MgCl₂, 100 mM NaCl, 1 mM DTT and 2 mM GDP (pH 7.7). Slides were subsequently incubated, for 2 h, in the same buffer containing adenosine deaminase (10 mU/ml) with[^14]S[^35]GTPγS (0.04 nM) and consecutive sections were co-incubated with (+)-8-OH-DPAT (10⁻⁴ M) or buspirone (10⁻⁴ M) alone or in the presence of the selective 5-HT₁A antagonist WAY 100635 (10⁻⁸ M). Non-specific binding was determined in the presence of 10 μM GTPγS. After incubation, the membrane suspension was solubilized for 30 min on ice with 1% Igepal, 0.5% sodium deoxycholate, 0.1% SDS, 2.5 mM Chaps, 0.1 mM phenylmethylsulfonylfluoride, 0.01 μM aprotinin, 1 μg/ml leupeptin, 1 μg/ml pepstatin, 1 μl/ml antipain, chymostatin. Next, 15 μl of specific rabbit antibody anti-Gαᵢ3, anti-Gαₒ, anti-Gαᵢ2, anti-Gαᵢ2, anti-Gαᵢ₃, anti-Gαᵢ₅ were incubated with the solubilized membranes for 45 min at room temperature, and 100 μl of GB-Sepharose beads were added and incubated for 3 h at room temperature. After three washes with 1 ml PBS the Sepharose beads were pelleted and the entrapped radioactivity was counted in 4 ml scintillation cocktail in a β counter (Beckman LS 600 IC, Beckman Instruments Inc., USA).

Antibody specificity was confirmed in our experimental conditions by Western blot.

**AC assay**

Fifty microlitres of freshly prepared DRN membranes (tissue pooled together from five rats, ~50 μg protein) were incubated for 5 min a 37 °C in 250 μl of 80 mM Tris–HCl (pH 7.4), 100 mM NaCl, 2 mM MgCl₂, 0.2 mM EGTA, 1 mM EDTA, 60 mM sucrose, 10 μM GTP, 1 mM DTT, 0.5 mM ascorbic acid, 0.5 μM 3-isobutylmethylxanthine, 5 μM phosphocreatine, 50 U/ml creatine phosphokinase and 5 U/ml myokinase and either water (basal AC activity), 5 μM forskolin (forskolin-stimulated cAMP accumulation), or increased concentrations of (+)-8-hydroxy-2-di-n-propylamino-tetralin (+)-8-OH-DPAT (10⁻⁹–10⁻⁴ M) or buspirone (10⁻⁸–10⁻⁴ M) in the presence of 5 μM forskolin. The enzymatic reaction was started by addition of ATP to a final concentration of 200 μM. The mixture was then incubated (10 min at 37 °C) and the reaction was rapidly terminated by 5 min incubation at 100 °C. The samples were centrifuged (13000 g, 5 min) and cAMP accumulation was quantified in 50 μl supernatant aliquots by using a[^3]H-cAMP commercial kit (TRK 432, Amersham Signal Transduction Assays; GE Healthcare UK Ltd, UK). The assays were performed in triplicate.

**Protein determination**

Membrane protein concentrations were determined by using the Bio-Rad Protein Assay kit (Bio-Rad, Germany) using BSA as the standard.
Data analysis

Autoradiograms were analysed and quantified using a computerized image analysis system (Scion Image, Scion Corporation, USA). For [35S]GTPγS experiments the data are presented as comparison of percent stimulation scores vs. basal binding.

Data analysis of agonist individual concentration–effect curves from cAMP assays were conducted by nonlinear regression using GraphPad Prism in order to estimate the theoretical maximal effect (Iₘₐₓ) and the potency (IC₅₀) of the 5-HT₁A agonists. IC₅₀ values were normalized as –log IC₅₀ (pIC₅₀) for comparison. Statistical comparison of agonist concentration–response curves was made using sum of squares F test. Differences in the agonist-dependent immunoprecipitation of each G-protein subunit was obtained by using one-way ANOVA with Newman–Keuls post-hoc tests. Differences were taken as statistically significant when p < 0.05.

Results

5-HT₁A receptor-dependent [35S]GTPγS binding to rat brain sections

Basal [35S]GTPγS binding was heterogeneous in rat brain, with relatively higher levels in DRN with regard to other areas such as hippocampus (dentate gyrus and CA1 field) or entorhinal cortex. Basal [35S]GTPγS binding levels in rat DRN were 597.3 ± 29.4 (nCi/g tissue). The co-incubation with the 5-HT₁A agonists (+)-8-OH-DPAT (10 μM) and buspirone (10 μM) induced a high stimulation of specific [35S]GTPγS binding in DRN [+42.8 ± 6.9% for (+)-8-OH-DPAT and +26.8 ± 6.3% for buspirone] (Fig. 1).

In agreement with previous studies, the selective 5-HT₁A antagonist WAY 100635 (10 μM) blocked the effect of (+)-8-OH-DPAT and buspirone on stimulation of [35S]GTPγS binding (Fig. 1).

Immunoprecipitation of Ga proteins labelled by [35S]GTPγS in soluble extracts from (+)-8-OH-DPAT- or buspirone-treated DRN membranes

We used GB-Sepharose beads to bind immunoprecipitates of Ga subunits labelled with [35S]GTPγS from DRN 5-HT₁A receptor/G-protein complexes, after incubation with either (+)-8-OH-DPAT or buspirone (10 μM) an increase in [35S]GTPγS binding over the basal value was observed for Ga₀, Ga₁₄ and Ga₁₃ protein subunits but not for Ga₃, Ga₅ or Ga₁₃ subunits. A significant (+)-8-OH-DPAT-induced [35S]GTPγS labelling of immunoprecipitates was obtained with anti-Ga₃ (132.0 ± 5.9%, p < 0.05, Student–Newman–Keuls test after ANOVA) antibody, but not with anti-Ga₀, anti-Ga₁₄, anti-Ga₁₃, anti-Ga₅ or anti-Ga₁₃ antibodies (Fig. 2). In contrast, significant buspirone-induced [35S]GTPγS labelling of immunoprecipitates was obtained with anti-Ga₀ (150.0 ± 3.6%, p < 0.01), anti-Ga₁₄ (132.0 ± 3.2%, p < 0.01) anti-Ga₃ (129.0 ± 2.3%, p < 0.05) antibodies but not with anti-Ga₁₃, anti-Ga₅ or anti-Ga₁₃. The selective 5-HT₁A antagonist WAY 100635 (10 μM) blocked both (+)-8-OH-DPAT and buspirone-induced [35S]GTPγS labelling of G-protein subunits (data not shown).

5-HT₁A receptor-mediated inhibition of AC

The selective 5-HT₁A agonist (+)-8-OH-DPAT (10⁻⁹ – 10⁻⁴ M) failed to significantly modify the level of forskolin-stimulated cAMP accumulation in rat DRN, although a slight but not significant increase was observed (Eₘₐₓ +10.6 ± 1.99; Fig. 3a). As (+)-OH-DPAT also binds to 5-HT₇ receptors (coupled to stimulatory Gₛ proteins), the possible influence of this receptor subtype was also studied. The presence of the selective 5-HT₇ receptor antagonist SB 269970 (10⁻⁵ M) did not modify the response of cAMP accumulation
to increasing concentrations of (+)-8-OH-DPAT in this rat brain region (Fig. 3a).

In contrast to (+)-8-OH-DPAT, buspirone induced a concentration-dependent decrease in forskolin-stimulated cAMP accumulation (Fig. 3b) with a maximum inhibition of 30.8 ± 4.8% and a pIC50 of 5.95 ± 0.46. This buspirone-mediated inhibition of cAMP accumulation was completely blocked by the 5-HT1A antagonist WAY100635 (F = 136.9, p < 0.001, sum of squares F test).

Discussion

We here report the existence of selective agonist-dependent signalling output for 5-HT1A receptors in the rat DRN, an area of special relevance for 5-HT neurotransmission, as these receptors regulate the firing of serotonergic neurons (Sprouse & Aghajanian, 1987). We demonstrate the existence of 5-HT1A receptor-dependent inhibition of AC activity in this area, a transductional response which has not been previously well identified in the rat. In addition, this is the first demonstration of agonist-directed trafficking of 5-HT1A receptors in native rat brain tissue.

Agonist-directed trafficking has been previously suggested in heterologously expressed 5-HT1A receptors (Heusler et al., 2005).

It has been shown that 5-HT1A receptors can physically couple to multiple distinct Ga proteins in mammalian cell membranes. It has also been suggested that functionality of these receptors inducing AC inhibition may be mainly mediated by Ga2 and Ga3, and Ga1 (Raymond et al., 1993). Furthermore, the existence of regional differences in the coupling of 5-HT1A receptors to the distinct subtypes of Ga proteins has been recently well demonstrated (Mannoury la Cour et al., 2006), under activation with 5-CT, 5-HT1A receptors interact with Ga1 and Ga3 in the cerebral cortex, mainly with Ga3, in the hippocampus, with Ga1 in the anterior raphe and with both Ga1 and Ga2 in the hypothalamus.

We have used agonist-induced [35S]GTPγS labelling (Harrison & Traynor, 2003) in tissue sections (autoradiography) at the level of midbrain to visualize, in a comparative way, the level of activation of the total population of Ga proteins in DRN by two different agonists. As this procedure does not allow examination of coupling of GPCRs to individual Ga subunits, we performed the recently developed G-protein antibody-capture assay coupled to Sepharose beads or to scintillation proximity assay (SPA) detection in order to study the activation patterns for the different G-protein subunits (Newman-Tancredi et al., 2002). In agreement with previous data obtained in both recombinant systems (Bertin et al., 1992; Garnovskaya et al., 1997; Raymond et al., 1993) and rat brain (Mannoury la Cour, 2006), our results show that native 5-HT1A receptors at DRN mainly couple to Ga1 subunit, regardless of the agonist used. In contrast, coupling of these receptors to Ga2 or Ga3 subunits seems to depend on the agonist assayed. Coupling of 5-HT1A receptors to Ga2 in vivo has been reported to result in inhibition of AC and increase in intracellular Ca2+ concentration (Albert et al., 1996; Raymond et al., 1993). In this regard, it is noteworthy that the agonist (+)-8-OH-DPAT was not able to couple to Ga2 subunits in DRN and did not inhibit AC activity. 5-CT, a drug that similarly to 8-OH-DPAT, behaves as a full agonist on all populations of 5-HT1A receptors, has also been reported to be unable to induce both coupling of the receptor to the Ga2 subunit (Mannoury la Cour et al., 2001) and inhibition of AC activity (Clarke et al., 1996) at the DRN level. In contrast, our results demonstrate that buspirone, a drug presenting a clear profile of functional heterogeneity at 5-HT1A receptors, induces both coupling of 5-HT1A receptor to Ga2, Ga1 and Ga3 Ga-protein
subunits and inhibition of signalling through cAMP second-messenger cascade. From these results it is tempting to suggest that 5-HT$_{1A}$ receptor-dependent AC inhibition in DRN might predominantly be related to G$_{ai2}$ subunit-mediated signalling. In contrast, G$_{ai3}$ and G$_{ao}$ may be implicated in channel activity regulation (reviewed in Raymond et al. 1999). In a similar context, Newman-Tancredi and colleagues have recently shown the existence of evidence for signal transduction and functional selectivity at 5-HT$_{1A}$ receptors, comparing the responses induced by several agonists (Newman-Tancredi et al. 2009). 5-HT$_{7}$ receptors are also present at DRN and their activation results in an increase of cAMP (Duncan & Davis, 2005). Taking into account that 8-OH-DPAT also binds with high affinity to this receptor subtype, it might be possible that their activation would mask a 5-HT$_{1A}$ receptor-dependent cAMP modification induced by this agonist: this is not the case, as in the presence of the selective 5-HT$_{7}$ receptor antagonist SB 269970, the lack of effect of (+)-8-OH-DPAT in rat DRN was still observed.

The existence of heterogeneity in the activation of transductional mechanisms following stimulation of 5-HT$_{1A}$ receptors is well documented. On the other hand, the preferential localization of these receptors over cell bodies and dendrites of serotonergic neurons at raphe nuclei has been widely demonstrated (Sprouse & Aghajanian, 1987). In this regard, our results strongly suggest the existence, at the rat DRN level, of drug-selective activation of specific G-protein subunits, thus supporting the agonist-directed trafficking of receptor signalling theory (Kenakin, 1995). Our results allow the re-interpretation of the different behaviour of buspirone at pre- and post-synaptic 5-HT$_{1A}$ receptors (full vs. partial agonism), although the existence of a large receptor reserve among auto-receptors in the raphe nuclei has been mentioned as a possible reason, it could be suggested that the agonist-dependent differential coupling to G-protein subunits throughout the brain would better explain the differences in efficacy.

In contrast to our results in rat DRN, in heterologous expression systems, such as CHO cells, both full 5-HT$_{1A}$ receptor agonists (5-HT and 8-OH-DPAT) and partial agonists (rauwolscine and ipsapirone) have been reported to induce labelling of G$_{ai2}$ and G$_{ai3}$ proteins with $[^{32}P]AA$-GTP, the level of isotopic incorporation being lower for the latter drugs (Gettys et al. 1994). These differences could be due to the fact that, in heterologous expression systems, the environmental conditions for the receptor and G-protein subunits, including level of surface expression, are completely different from those occurring in native

![Fig. 3](image-url)
Agonist-dependent 5-HT$_{1A}$ signalling in DRN

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They also emphasize the interest in performing these kind of studies in native tissue, when conclusions for therapeutic activity of drugs are to be obtained.

Several functional results suggest a differential activation of transductional mechanisms by 5-HT$_{1A}$ receptor agonists. In this regard, 5-CT and 8-OH-DPAT have been demonstrated to induce a marked hypothermia and a flattening of body posture. In contrast, no significant reduction in core temperature or induction of a marked flattening of posture has been observed with buspirone (Higgins, 1988). Furthermore, differences in the degree of activation of diverse transductional mechanisms have been implicated in the origin of the regional differences in 5-HT$_{1A}$ receptor/G-protein coupling observed (DRN vs. telencephalic areas) following SSRI treatment in rats, as well as in 5-HT$^{-/-}$ mice. (Castro et al. 2003; Fabre et al. 2000; Hensler, 2002; Le Poul et al. 2000; Mannoury la Cour et al. 2001).

Regarding the diversity of transductional responses associated to 5-HT$_{1A}$ receptors, it must be taken into account that a species-sensitive variation in the functional coupling to AC has been observed in DRN between rat and humans: in the rat, 5-HT$_{1A}$ receptor activation by 8-OH-DPAT does not result in AC inhibition (Clarke et al. 1996; Johnson et al. 1997, present study), while activation by buspirone does modify signalling of 5-HT$_{1A}$ receptors through cAMP (present study). In contrast, inhibition of AC signalling through activation of 5-HT$_{1A}$ receptors by both 8-OH-DPAT (Palego et al. 1999, 2000) and buspirone (Marazziti et al. 2002) has been reported in humans. The molecular basis of this species variation is not clear at the present time.

In conclusion, our results demonstrate the existence of agonist-directed trafficking for 5-HT$_{1A}$ receptor signalling in rat native brain tissue, helping to explain the important differences in pharmacological efficacy found for some agonists, such as buspirone: these findings open new perspectives for the differential analysis of pre- vs. post-synaptic 5-HT$_{1A}$ receptor-associated responses. Moreover, this agonist-dependent differential coupling to G-protein subunits can contribute to the understanding of the differences found in the adaptive changes of 5-HT$_{1A}$ receptor functionality caused by chronic administration of 5-HT-acting drugs, as it is the case with antidepressants.

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Statement of Interest

Over the past two years Angel Pazos has received compensation from FAES FARMA SA.

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