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ARTICLE

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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 adenylyl 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 Gα subtypes ([^{35}S]GTPγS labelling and immunoprecipitation) involved and the subsequent changes in cAMP formation. A significant increase (32%, $p<0.05$) in (+)8-OH-DPAT-induced [^{35}S]GTPγS labelling of immunoprecipitates was obtained with anti-Gα_{i3} antibodies but not with anti-Gα_o, anti-Gα_{i1}, anti-Gα_{i2}, anti-Gα_z or anti-Gα_s antibodies. In contrast, in the presence of buspirone, significant [^{35}S]GTPγS labelling of immunoprecipitates was obtained with anti-Gα_{i3} (50%, $p<0.01$), anti-Gα_o (32%, $p<0.01$) and anti-Gα_{i2} (29%, $p<0.05$) antibodies, without any labelling with anti-Gα_{i1}, anti-Gα_z or anti-Gα_s. 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±4.9, pIC_{50} 5.95±0.46). 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_{i1}, G_{i2}, G_{i3} and G_o, and pertussis toxin-insensitive G_z proteins (reviewed in Raymond *et al*.

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1999). In heterologous recombinant systems, 5-HT_{1A} receptors preferentially interact with $G\alpha_{i3}$ subunits, and, to a less extent, with other members of the group: $G\alpha_{i2} < G\alpha_o < G\alpha_{i1} < G\alpha_z$ (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_{1A} receptors with $G\alpha_q$ and $G\alpha_s$ is very weak (Raymond *et al*. 1993). In rat brain tissue, a regional pattern of preferential coupling to $G\alpha$ 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_{1A} receptors to G_i/G_o 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 Cβ and mitogen-activated protein kinase (MAPK) (Raymond *et al*. 2001).

5-HT_{1A} autoreceptors localized over raphe nuclei play a critical role in the regulation of the functionality of 5-HT neurotransmission: in the rat DRN, activation of 5-HT_{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_{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_{1A} receptors have been recently published (Newman-Tancredi *et al*. 2009).

It is noteworthy that the pharmacological profile of different 5-HT_{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_{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_{1A} receptor activation of specific subtypes of $G\alpha$ 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 [^{35}S]GTPγS 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 *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

[^{35}S]GTPγS (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 (GTPγS), adenosine 5′-triphosphate disodium salt (ATP), ethylenediaminetetraacetic acid (EDTA), ethylene glycol-bis (β-aminoethyl 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, chimostatin, sodium deoxicholate, igepal, (+)8-hydroxy-2-di-*n*-propylamino-tetralin [(+)8-OH-DPAT] and [*N*-[2-(4-(2-methoxyphenyl)-1-piperazinyl] ethyl]-*N*-2-pyridinyl-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-$G\alpha_{i1}$ (*sc-391*), anti-$G\alpha_{i2}$ (*sc-7276*), anti-$G\alpha_{i3}$ (*sc-262*), anti-$G\alpha_{iz}$ (*sc-388*) and anti-$G\alpha_o$ (*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.

[^{35}S]GTPγS autoradiography

Labelling of brain sections with [^{35}S]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 $MgCl_2$, 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 [^{35}S]GTPγS (0.04 nM) and consecutive sections were co-incubated with (+)8-OH-DPAT (10^{-5} M) or buspirone (10^{-5} M) alone or in the presence of the selective 5-HT$_{1A}$ antagonist WAY 100635 (10^{-5} M). Non-specific binding was determined in the presence of 10 μM GTPγS.

After incubation, the sections were washed twice for 15 min in cold 50 mM Tris–HCl buffer (pH 7.4) at 4 °C, rinsed in distilled cold water and then dried under a cold air stream. Sections were exposed to autoradiographic film (Biomax MR, Spain) together with ^{14}C microscales at 4 °C for 2 d.

Membrane preparation

DRN tissues were homogenized using a glass-Teflon driver potter (Heidolph Instruments, GmbH & Co., Germany) in ice-cold buffer (20 mM Tris–HCl, 1 mM EGTA, 5 mM EDTA, 300 mM sucrose, 5 mM DTT, 25 mg/ml leupeptin) (pH 7.4) and centrifuged (1500 ***g***) for 5 min at 4 °C. The supernatants were pelleted (13000 ***g***) for 15 min and used for AC assay or immunoprecipitation of [^{35}S]GTPγS-labelled Gα subunits.

Immunoprecipitation of [^{35}S]GTPγS-labelled Gα subunits

For immunoprecipitation experiments, membranes from DRN (tissue pooled together from five rats, for each specific Gα protein) were prepared as described in the previous section. The final pellet was resuspended in assay buffer [50 mM Tris–HCl (pH 7.4), 3 mM $MgCl_2$, 1 mM EGTA, 1 mM DTT, 100 mM NaCl, 50 μM GDP]. Then 50 μl of membranes (~250 μg protein) were incubated in a final 100 μl assay volume with 2 nM [^{35}S]GTPγS and 10^{-5} M (+)8-OH-DPAT or buspirone for 30 min at 30 °C. 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 M aprotinin, 1 μg/ml leupetin, 1 μg/ml pestatin, 1 μl/ml antipain, chimostatin. Next, 15 μl of specific rabbit antibody anti-Gα_{i1}, anti-Gα_{i2}, anti-Gα_{i3}, anti-Gα_z, anti-Gα_o 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 $MgCl_2$, 0.2 mM EGTA, 1 mM EDTA, 60 mM sucrose, 10 μM GTP, 1 mM DTT, 0.5 mM ascorbic acid, 0.5 mM 3-isobutylmethylxanthine, 5 mM 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 [^{35}S]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 (I_{max}) and the potency (IC_{50}) of the 5-HT$_{1A}$ agonists. IC_{50} values were normalized as –log IC_{50} (pIC_{50}) 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-HT$_{1A}$ receptor-dependent [^{35}S]GTPγS binding to rat brain sections

Basal [^{35}S]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 [^{35}S]GTPγS binding levels in rat DRN were 597.3 ± 29.4 (nCi/g tissue). The co-incubation with the 5-HT$_{1A}$ agonists (+)8-OH-DPAT (10 μM) and buspirone (10 μM) induced a high stimulation of specific [^{35}S]GTPγS binding in DRN [$+42.8\pm6.9$% for (+)8-OH-DPAT and $+26.8\pm6.3$% for buspirone] (Fig. 1).

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

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

We used GB-Sepharose beads to bind immunoprecipitates of Gα subunits labelled with [^{35}S]GTPγS from DRN 5-HT$_{1A}$ receptor/G-protein complexes, after incubation with either (+)8-OH-DPAT or buspirone (10 μM) an increase in [^{35}S]GTPγS binding over the basal value was observed for Gα_{o}, Gα_{i2} and Gα_{i3} protein subunits but not for Gα_{i1}, Gα_{z} or Gα_{s} subunits. A significant (+)8-OH-DPAT-induced [^{35}S]GTPγS labelling of immunoprecipitates was obtained with anti-Gα_{i3} (132.0 ± 5.9%, $p<0.05$, Student–Newman–Keuls test after ANOVA) antibody, but not with anti-Gα_{o}, anti-Gα_{i1}, anti-Gα_{i2}, anti-Gα_{z} or anti-Gα_{s} antibodies (Fig. 2). In contrast, significant buspirone-induced [^{35}S]GTPγS labelling of immunoprecipitates was obtained with anti-Gα_{i3} (150.0 ± 3.6%, $p<0.01$), anti-Gα_{o} (132.0 ± 3.2%, $p<0.01$) anti-Gα_{i2} (129.0 ± 2.3%, $p<0.05$) antibodies but not with anti-Gα_{i1}, anti-Gα_{z} or anti-Gα_{s}. The selective 5-HT$_{1A}$ antagonist WAY 100635 (10 μM) blocked both (+)8-OH-DPAT and buspirone-induced [^{35}S]GTPγS labelling of G-protein subunits (data not shown).

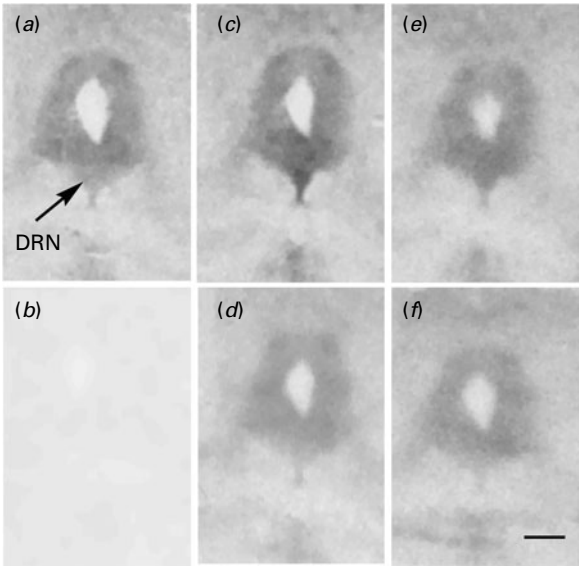

Fig. 1. Representative autoradiograms of [^{35}S]GTPγS binding in coronal sections of rat brain at the midbrain level: (*a*) basal binding; (*b*) non-specific binding; (*c*) binding in the presence of (+)8-OH-DPAT (10^{-5} M); (*d*) binding in the presence of (+)8-OH-DPAT (10^{-5} M) + WAY 100635 (10^{-5} M); (*e*) binding in the presence of buspirone (10^{-5} M) and (*f*) binding in the presence of buspirone (10^{-5} M) + WAY 100635 (10^{-5} M). DRN, Dosal raphe nucleus (bar, 2 mm).

5-HT$_{1A}$ receptor-mediated inhibition of AC

The selective 5-HT$_{1A}$ 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 (E_{max} $+10.6\pm1.99$; Fig. 3*a*). As (+)8-OH-DPAT also binds to 5-HT$_{7}$ receptors (coupled to stimulatory G_s proteins), the possible influence of this receptor subtype was also studied. The presence of the selective 5-HT$_{7}$ receptor antagonist SB 269970 (10^{-5} M) did not modify the response of cAMP accumulation

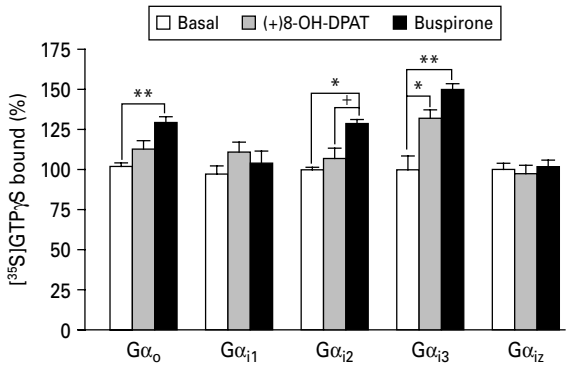

Fig. 2. Immunoprecipitation of G-protein subunit labelled with [^{35}S]GTPγS from 5-HT$_{1A}$ receptor/G-protein complexes solubilized from dorsal raphe nucleus membranes after incubation with 10 μM (+)8-OH-DPAT or 10 μM buspirone. [^{35}S]-labelled Gα subunits were immunoprecipitated then bound onto protein GB-Sepharose beads, as described in the Materials and methods section. Results are expressed as percent of basal labelling with [^{35}S]GTPγS. Each bar is the mean $\pm$ S.E.M. of triplicate determinations in 6–8 independent experiments. A significant increase in [^{35}S]GTPγS over the basal value (100%) was observed for Gα_o ($F=6.6$, $p<0.01$), Gα_{i2} ($F=6.0$, $p<0.05$), and Gα_{i3} ($F=8.4$, $p<0.01$), but not for Gα_{i1} ($F=0.5$, n.s.) or Gα_z ($F=0.7$, n.s.). *F*: one-way ANOVA; * $p<0.05$, ** $p<0.01$ *vs.* the basal binding and $^+$ $p<0.05$ *vs.* (+)8-OH-DPAT by Student–Newman–Keuls *post-hoc* test.

to increasing concentrations of (+)8-OH-DPAT in this rat brain region (Fig. 3*a*).

In contrast to (+)8-OH-DPAT, buspirone induced a concentration-dependent decrease in forskolin-stimulated cAMP accumulation (Fig. 3*b*) with a maximum inhibition of 30.8 $\pm$ 4.8% and a pIC$_{50}$ of 5.95 $\pm$ 0.46. This buspirone-mediated inhibition of cAMP accumulation was completely blocked by the 5-HT$_{1A}$ antagonist WAY 100635 ($F=136.9$, $p<0.001$, sum of squares *F* test).

Discussion

We here report the existence of selective agonist-dependent signalling output for 5-HT$_{1A}$ 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-HT$_{1A}$ 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-HT$_{1A}$ receptors in native rat brain tissue. Agonist-directed trafficking has been previously suggested in heterologously expressed 5-HT$_{1A}$ receptors (Heusler *et al*. 2005).

It has been shown that 5-HT$_{1A}$ receptors can physically couple to multiple distinct Gα_i proteins in mammalian cell membranes. It has also been suggested that functionality of these receptors inducing AC inhibition may be mainly mediated by Gα_{i3} and Gα_{i2}, and Gα_{i1} (Raymond *et al*. 1993). Furthermore, the existence of regional differences in the coupling of 5-HT$_{1A}$ receptors to the distinct subtypes of G proteins has been recently well demonstrated (Mannoury la Cour *et al*. 2006), under activation with 5-CT, 5-HT$_{1A}$ receptors interact with Gα_o and Gα_{i3} in the cerebral cortex, mainly with Gα_o in the hippocampus, with Gα_{i3} in the anterior raphe and with both Gα_{i1} and Gα_z in the hypothalamus.

We have used agonist-induced [^{35}S]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 G proteins in DRN by two different agonists. As this procedure does not allow examination of coupling of GPCRs to individual Gα 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-HT$_{1A}$ receptors at DRN mainly couple to Gα_{i3} subunit, regardless of the agonist used. In contrast, coupling of these receptors to Gα_{i2} or Gα_o subunits seems to depend on the agonist assayed. Coupling of 5-HT$_{1A}$ receptors to Gα_{i2} *in vivo* has been reported to result in inhibition of AC and increase in intracellular Ca^{2+} 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 Gα_{i2} 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-HT$_{1A}$ receptors, has also been reported to be unable to induce both coupling of the receptor to the Gα_{i2} 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-HT$_{1A}$ receptors, induces both coupling of 5-HT$_{1A}$ receptor to Gα_o, Gα_{i2} and Gα_{i3} G-protein

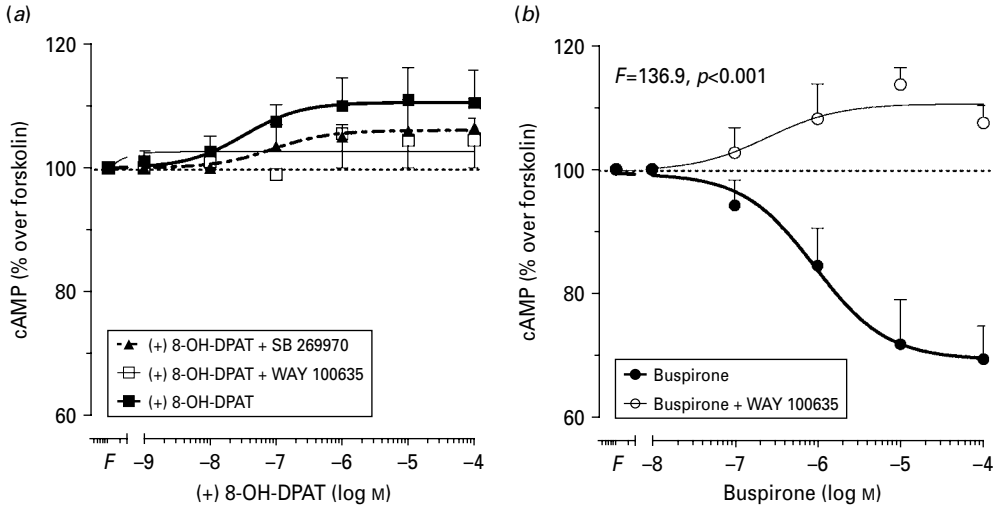

Fig. 3. Inhibition of forskolin-stimulated adenylyl cyclase (AC) activation by the 5-HT_{1A} receptor agonist: (*a*) concentration-dependent (+)8-OH-DPAT ($10^{-9}-10^{-4}$ M) modulation of forskolin-stimulated AC activity alone and in the presence of the selective 5-HT_{1A} receptor antagonist WAY 100635 (10^{-5} M), or in the presence of the selective 5-HT_7 receptor antagonist SB 269970 (10^{-5} M). (*b*) concentration-dependent inhibition of forskolin-stimulated AC activity by buspirone ($10^{-8}-10^{-4}$ M) alone and in the presence of the selective 5-HT_{1A} receptor antagonist (10^{-5} M). Percentages of inhibition were calculated for each agonist concentration relative to forskolin-stimulated cAMP levels. Data are mean ± S.E.M. of six independent experiments. *F*: sum of squares *F* test, buspirone *vs*. buspirone + WAY 100635.

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 $G\alpha_{i2}$ subunit-mediated signalling. In contrast, $G\alpha_{i3}$ and $G\alpha_o$ 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 autoreceptors 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 $G\alpha_{i2}$ and $G\alpha_{i3}$ 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

tissue. 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-HTT$^{-/-}$ 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 adaptative 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

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