Expression of $\alpha_1$-adrenergic receptors in rat prefrontal cortex: cellular co-localization with 5-HT$_2A$ receptors

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Abstract

The prefrontal cortex (PFC) is involved in behavioural control and cognitive processes that are altered in schizophrenia. The brainstem monoaminergic systems control PFC function, yet the cells/networks involved are not fully known. Serotonin (5-HT) and norepinephrine (NE) increase PFC neuronal activity through the activation of $\alpha_1$-adrenergic receptors ($\alpha_1$ARs) and 5-HT$_2A$ receptors (5-HT$_2A$Rs), respectively. Neurochemical and behavioural interactions between these receptors have been reported. Further, classical and atypical antipsychotic drugs share in vitro affinity for $\alpha_1$ARs while having preferential affinity for D$_2$ and 5-HT$_2A$Rs, respectively. Using double in situ hybridization we examined the cellular expression of $\alpha_1$ARs in pyramidal (vGluT1-positive) and GABAergic (GAD$^+$-positive) neurons in rat PFC and their co-localization with 5-HT$_2A$Rs. $\alpha_1$ARs are expressed by a high proportion of pyramidal (59–85%) and GABAergic (52–79%) neurons. The expression in pyramidal neurons exhibited a dorsoventral gradient, with a lower percentage of $\alpha_1$AR-positive neurons in infralimbic cortex compared to anterior cingulate and prelimbic cortex. The expression of $\alpha_1$A, $\alpha_1$B and $\alpha_1$D adrenergic receptors was segregated in different layers and subdivisions. In all them there is a high co-expression with 5-HT$_2A$Rs (~80%). These observations indicate that NE controls the activity of most PFC pyramidal neurons via $\alpha_1$ARs, either directly or indirectly, via GABAergic interneurons. Antipsychotic drugs can thus modulate the activity of PFC via $\alpha_1$AR blockade. The high co-expression with 5-HT$_2A$Rs indicates a convergence of excitatory serotonergic and noradrenergic inputs onto the same neuronal populations. Moreover, atypical antipsychotics may exert a more powerful control of PFC function through the simultaneous blockade of $\alpha_1$ARs and 5-HT$_2A$Rs.

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Introduction

The prefrontal cortex (PFC) is critically involved in many higher brain functions, including cognition, attention and behavioural planning (Fuster, 1997; Miller & Cohen, 2001). Anatomical, cellular and neurochemical alterations of the PFC have been reported in schizophrenia (Harrison, 1999; Lewis & Lieberman, 2000; Lewis et al. 2005; Selemon & Goldman-Rakic, 1999; Weinberger et al. 2001).

The brainstem monoaminergic systems innervate the PFC and modulate the activity of memory networks and animal behaviour (Carli et al. 2006; Dalley et al. 2004; Ramos & Arnsten, 2007; Robbins, 2000; Roberts et al. 1994; Vijayraghavan et al. 2007; Winstanley et al. 2003).

Particular attention has been paid to the dopaminergic system, which finely tunes the activity of PFC neurons during spatial working memory tasks via D$_1$ receptors in a bell-shaped manner (Vijayraghavan et al. 2007; Williams & Goldman-Rakic, 1995).

Less is known of the role of norepinephrine (NE) and serotonin (5-HT) in PFC function. Hence, moderately elevated levels of NE acting on $\alpha_2$A-adrenergic receptors ($\alpha_2$AARs) improve cognitive performance whereas high NE release (e.g. as evoked by stress) impairs cognitive performance by activating $\alpha_1$ARs (Ramos & Arnsten, 2007). On the other hand, 5-HT$_2A$R blockade partly antagonizes the neuronal activation during a spatial working-memory task (Williams et al. 2002) and seratonin (5-HT) depletion in monkey orbitofrontal cortex impaired reversal learning (Clarke et al. 2004). Further, the local blockade of PFC 5-HT$_2A$Rs improves visuospatial attention and decreases impulsivity in rats (Winstanley et al. 2003).
Electrophysiological studies in rodents have shown that NE and 5-HT can modulate the activity of PFC neurons in vivo and in vitro (Aghajanian & Marek, 1997; Araneda & Andrade, 1991; Arvanov et al. 1999; Ashby et al. 1989; Férézou et al. 2002; McCormick et al. 1993; Puig et al. 2003, 2004, 2005; Tanaka & North, 1993; Zhang & Arsenault, 2005; Zhou & Hablitz, 1999). In particular, the excitatory actions of NE and 5-HT on PFC cells may be involved in the maintenance of activity patterns during working-memory tasks. These excitatory actions are mediated by α1ARs and 5-HT2A receptors, respectively (Aghajanian & Marek, 1997; Araneda & Andrade, 1991; Puig et al. 2003, 2005; Zhang & Arsenault, 2005). Additionally, 5-HT can excite GABAergic interneurons via 5-HT3 receptors (Gui et al. 2010; Puig et al. 2004).

The knowledge of the cellular and network basis of NE and 5-HT modulation of PFC activity is also important in clarifying the mechanism of action of antipsychotic drugs. Hence, classical antipsychotics show high affinity for D2 receptors, whereas atypical antipsychotics show preferential affinity for 5-HT2A receptors vs. D2 receptors. Yet, all of them share high affinity for α1ARs (Arnt & Skarsfeldt, 1998; Bymaster et al. 1996; Meltzer, 1999) although the potential therapeutic role of α1AR blockade has been obscured by its involvement in cardiovascular side-effects.

Interestingly, functional interactions between 5-HT1A and α1ARs have been reported, which may be involved in the therapeutic activity of antipsychotic drugs. Hence, neurochemical and behavioural actions of 5-HT1AR agonists can be antagonized by 5-HT1A and α1AR blockade as well as by antipsychotic drugs (Amargós-Bosch et al. 2003; Bortolozzi et al. 2003; Dursun & Handley, 1996; Schreiber et al. 1995). Conversely, neurochemical effects of the α1AR agonist cirazoline in PFC can be reversed by prazosin, M100907 (a selective 5-HT1A receptor antagonist) and by antipsychotic drugs (Amargós-Bosch et al. 2003). 5-HT1A/α1AR interactions may also participate in the action of opiates and psychostimulants (Auclair et al. 2004).

The cellular/network basis of the above interactions is unknown. Previous studies have revealed the presence of 5-HT1A receptors in pyramidal and GABAergic neurons of rat PFC (Martín-Ruiz et al. 2001; Santana et al. 2004). However, the cellular expression of α1AR is presently unknown. Using double in situ hybridization, we performed a quantitative study on the expression of α1A, α1B and α1D adrenergic receptors in pyramidal vesicular glutamate transporter 1 (vGlut1)-positive and GABAergic (GAD65/67-positive) cells of rat PFC. Similarly, given the common signalling pathways shared by 5-HT1A and α1ARs (Gq/11 G proteins) (Bartrup & Newberry, 1994; Berg et al. 1998; Claro et al. 1993; Michel et al. 1993; Molinoff, 1984) and the above functional interactions, we examined the co-expression of both receptors in rat PFC using double in situ hybridization.

Materials and method

Tissue preparation

Male albino Wistar rats weighing 250–300 g (Iffa Credo, France) were used. Animals were kept in a controlled environment [12-h light–dark cycle (lights on 08:00 hours) and 22 ± 2 °C room temperature] with food and water provided ad libitum. Animal care followed the European Union regulations (OJ of EC L358/1 18/12/1986) and was approved by the local Institutional Animal Care and Use Committee. Rats were killed by decapitation and the brains rapidly removed, frozen on dry ice and stored at −20 °C. Tissue sections, 14-μm thick, were cut using a microtome-cryostat (HM500 OM; Microm, Germany), thaw-mounted onto 3-aminopropyltriethoxysilane- (APTS; Sigma, USA) coated slides and kept at −20 °C until use.

Hybridization probes

The oligodeoxyribonucleotide probes used were complementary to the following bases: 2–46 and 1395–1439 of the rat α1AAR mRNA (GenBank accession no. NM_017191.1); 365–409 and 2216–2260 of the rat α1BAR mRNA (GenBank accession no. NM_012563.1); 128–170, 180–224, 836–880, 939–987, 1301–1345 and 1380–1427 of the rat 5-HT2A mRNA (GenBank accession no. NM_017254.1), 514–558 of the isoform of the enzyme glutamate decarboxylase (GAD67) mRNA (GenBank accession no. NM_012563.1); 1600–1653 of the GAD65 mRNA (GenBank accession no. NM_017007.1); 127–171 and 1756–1800 of the vGlut1 mRNA (GenBank accession no. NM_053859.1).

All probes were synthesized and HPLC purified by Isogen Bioscience BV (Maarsden, The Netherlands) and labelled either with [33P] or with digoxigenin (Dig). Each receptor oligonucleotide was individually labelled (2 pmol) at the 3'-end either with [33P]dATP (> 2500 Ci/mmole; DuPont-NEN, USA) using terminal deoxynucleotidyl transferase (TdT, Calbiochem, USA). GAD and vGlut oligonucleotides (100 pmol) were non-radioactively labelled with TdT (Roche Diagnostics GmbH, Germany) and Dig-11-dUTP (Boehringer Mannheim). Oligonucleotides were purified by centrifugation using ProbeQuant™ G-50 micro columns (GE Healthcare UK Ltd, UK). All probes were tested for specificity prior to use.

In situ hybridization histochemistry procedure

The protocol for single- and double-label in situ hybridization has been published elsewhere (Serrats et al. 2003). Frozen tissue sections were first brought to room temperature, fixed for 20 min at 4 °C in 4% paraformaldehyde in phosphate-buffered saline (1×PBS: 8 mM NaH2PO4, 1.4 mM KH2PO4, 136 mM NaCl, 2.6 mM KCl), washed for 5 min in 3×PBS at room temperature,
twice for 5 min each in 1× PBS and incubated for 2 min at 21°C in a solution of predigested pronase (Calbiochem, USA) at a final concentration of 24 U/ml in 50 mM Tris–HCl (pH 7.5), 5 mM EDTA. The enzymatic activity was stopped by immersion for 30 s in 2 mg/ml glycine in 1× PBS. Tissues were finally rinsed in 1× PBS and dehydrated through a graded series of ethanol. For hybridization, the radioactively labelled and the non-radioactively labelled probes were digested in a solution containing 50% formamide, 4× SSC (1× SSC: 150 mM NaCl, 15 mM sodium citrate), 1× Denhardt’s solution (0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02% BSA), 10% dextran sulfate, 1% sarkosyl, 20 mM phosphate buffer (pH 7.0), 250 μg/ml yeast tRNA and 500 μg/ml salmon sperm DNA. The final concentrations of radioactive and Dig-labelled probes in the hybridization buffer were in the same range (~1.5 nM). Tissue sections were covered with hybridization solution containing the labelled probe(s), overlaid with Nescofilm coverslips (Bando Chemical Industries, Japan) and incubated overnight at 42°C in humidity boxes. Sections were then washed four times (45 min each) in a buffer containing 0.6 mM NaCl and 10 mM Tris–HCl (pH 7.5) at 60°C.

Development of radioactive and non-radioactive hybridization signal

Hybridized sections were treated as described by Landry et al. (2000) (see updated procedures in Santana et al. 2004, 2009). Briefly, after washing, the slides were immersed for 30 min in a buffer containing 0.1 mM Tris–HCl (pH 7.5), 1 mM NaCl, 2 mM MgCl₂ and 0.5% BSA (Sigma) and incubated overnight at 4°C in the same solution with alkaline-phosphate-conjugated anti-digoxigenin-F(ab) fragments (1:5000; Boehringer Mannheim, Germany). Afterwards, they were washed three times (10 min each) in the same buffer (without antibody) and twice in an alkaline buffer containing 0.1 mM Tris–HCl (pH 9.5), 0.1 mM NaCl and 5 mM MgCl₂. Alkaline phosphatase activity was developed by incubating the sections with 3.3 mg nitroblue tetrazolium and 3.3 mg bromochloroindolyl phosphate (Gibco BRL, USA) diluted in 10 mM alkaline buffer. The enzymatic reaction was blocked by extensive rinsing in the alkaline buffer containing 1 mM EDTA. The sections were then briefly dipped in 70% and 100% ethanol, air-dried and dipped into Ilford K5 nuclear emulsion (Ilford, UK) diluted 1:1 with distilled water. They were exposed in the dark at 4°C for 5 wk and finally developed in Kodak D19 (Kodak, USA) for 5 min and fixed in Ilford Hypam fixer (Ilford).

Analysis of the results

Tissue sections were examined in bright- and dark-field in a Wild 420 microscope (Leica, Germany) and in a Nikon Eclipse E1000 microscope (Nikon, Japan) equipped with bright- and dark-field condensers for transmitted light and with epi-illumination. Micrography was performed using a digital camera (DXM1200 3.0; Nikon) and analySIS software (Soft Imaging System GmbH, Germany). Bright-field images were captured with transmitted light. Dark-field images were captured with Darklite illuminator (Micro Video Instruments, USA). The figures were prepared for publication using Adobe Photoshop software (Adobe Software, USA). The cellular counting was performed with an Olympus AX70 stereo microscope using CAST software for stereological analysis, and an Olympus BX51 stereo microscope equipped with an Olympus microscope digital camera DP71 and Visiopharm Integrator System software (Olympus). Data for total α₁AR expression was obtained by hybridizing PFC sections with a combination of the oligonucleotides selective for α₁A, α₁B and α₁D adrenergic receptor mRNA. Co-expression data of vGluT1, GAD, or 5-HT₂A-R-positive cells (Dig-labelled cells) with α₁AR mRNA (or individual α₁A, α₁B and α₁D adrenergic receptor mRNA) was estimated by counting the number of Dig-labelled cellular profiles which also expressed α₁AR mRNA (radioactively labelled cells). Relative proportions were estimated by considering 100% of the Dig-labelled cells. Only cellular profiles showing 2-fold density of silver grains from background were considered to be double-labelled. Data are means ± S.E.M. of three rats (duplicate/triplicate sections per rat, see Figure legends).

Results

Expression of α₁AR in the rat PFC

The various subtypes of α₁AR mRNA were abundantly expressed in rat PFC, in particular α₁A and α₁D adrenergic receptors. Figure 1 shows the distribution of each individual α₁AR transcript. Several features can be recognized. First, virtually all subdivisions of the PFC contained cells expressing one or more α₁ARs. Second, most abundant transcripts were those corresponding to α₁A and α₁D adrenergic receptors, whereas the expression of α₁B AR was more moderate, particularly in the medial PFC (mPFC). Third, with few exceptions, the various α₁AR transcripts showed an almost non-overlapping regional distribution within the mPFC. This was particularly remarkable for α₁A and α₁D adrenergic receptors. The former transcript was densely expressed in deep layers (VIA, VIb) of the medial, dorsal and lateral (agranular insular) PFC and the claustrum as well as in ventral areas, such as orbital and piriform cortices and the tenia tecta (TT). In contrast, the α₁D AR transcript was particularly abundant in intermediate layers (layers II–III, outer layer V) in medial and dorsolateral aspects of the PFC as well as in orbital cortex and had a much more restricted expression in TT and piriform cortex. In addition to this differential regional distribution (see Fig. 1a, c), there was a common expression of both receptors in superficial layers of medial and dorsolateral aspects of the PFC.
The density of the $\alpha_{1B}$AR transcript was lower than that of $\alpha_{1A}$ARs and $\alpha_{1D}$ARs. Moreover, its regional distribution was more restricted, being localized to dorsal and lateral aspects of PFC (layers III–V), as well as orbital and piriform cortices.

Expression of $\alpha_A$ARs in pyramidal and GABAergic neurons of the rat PFC

In double in situ hybridization experiments, we observed the presence of all three types of $\alpha_A$ARs in pyramidal (vGluT1-positive) and GABAergic (GAD$_{67}$-positive) neurons. Figures 2–4 show representative examples of the presence of $\alpha_{1A}$, $\alpha_{1B}$, and $\alpha_{1D}$ adrenergic receptor transcripts in pyramidal and GABAergic neurons in various layers of the mPFC. Note the abundance of pyramidal and GABAergic neurons expressing $\alpha_{1A}$ and $\alpha_{1D}$ adrenergic receptors. Table 1 shows the proportion of pyramidal and GABAergic neurons in the cingulate, prefrontal, and infralimbic areas of the mPFC expressing $\alpha_A$ARs.

The proportion of pyramidal neurons expressing $\alpha_A$ARs in the different cortical layers and areas of the mPFC was very high (59–85%), being maximal in layers II–III and V of the cingulate and prefrontal areas (71–85%) (Table 1). There was a dorsoventral gradient in the proportion of pyramidal cells expressing $\alpha_A$ARs, with a greater percentage in the anterior cingulate and a lower proportion in the infralimbic area, particularly in layer VI (Table 1).

Similarly, a very high proportion of GABAergic neurons also expressed $\alpha_A$ARs (52–79%). GABAergic cells expressing each of the three $\alpha_A$AR subtypes were found in different cortical layers, including layer I. Figures 2–4 show representative examples of GABAergic cells expressing $\alpha_{1A}$, $\alpha_{1B}$, and $\alpha_{1D}$ adrenergic receptors in the mPFC. Interestingly, unlike the other $\alpha_A$AR subtypes, some cells in superficial layers of the prefrontal area exhibited a remarkably high level of the $\alpha_{1B}$AR transcript (data not shown).

Further analysis of the regional expression of the different $\alpha_A$AR subtypes revealed that pyramidal neurons expressing $\alpha_{1A}$ARs were more abundantly localized in deep layers (V–VI) in the three mPFC subdivisions (ACAd, PL, ILA) (layer factor: $F_{1,12} = 35.7, p < 0.0001$; region factor: n.s.; two-way ANOVA). No effect of layer and region was found for $\alpha_{1A}$ARs expressed in GABAergic neurons. In contrast, pyramidal neurons expressing $\alpha_{1D}$ARs were mainly localized in superficial layers (II–III) in the same regions (layer factor: $F_{1,12} = 453.7, p < 0.0001$; region factor: $F_{1,12} = 35.7, p < 0.05$;
Fig. 2. Top row (A1–A3). High magnification photomicrographs showing the presence of $\alpha_{1A}$ adrenergic receptor mRNA (detected by hybridization with $^{33}$P-labelled oligonucleotides, seen as silver grains) in layer V pyramidal cells of the prelimbic area. Pyramidal neurons were identified by the presence of vesicular glutamate transporter 1 mRNA (detected by hybridization with Dig-labelled oligonucleotides, seen as dark precipitates). Red arrowheads indicate cells positive for both transcripts. For the sake of simplicity, only few cells of each type are shown. The majority of glutamatergic cells expressed $\alpha_{1A}$ adrenergic receptor mRNA, as denoted by the double labelling. Bottom row (B1–B3). $\alpha_{1A}$ adrenergic receptor mRNA was also found in GABAergic (GAD$^{65/67}$-positive) cells throughout the prefrontal cortex (Dig-labelled oligonucleotides, dark precipitates). Panels B1 and B2 show the presence of $\alpha_{1A}$ adrenergic receptor mRNA in some GABAergic cells of layer I and deep layers, of the prelimbic area, respectively, in the medial prefrontal cortex. Panel B3 shows double-labelled cells in the prelimbic area at higher magnification. Bar size, 20 $\mu$m.

Fig. 3. Top row (A1–A3). High magnification photomicrographs showing the presence of $\alpha_{1B}$ adrenergic receptor (AR) mRNA ($^{33}$P-labelled oligonucleotides, silver grains) in pyramidal cells, identified by the presence of vesicular glutamate transporter 1 mRNA (Dig-labelled oligonucleotides, dark precipitates). Panel A1 corresponds to layers II–III of anterior cingulate area. Panel A2 shows double-labelled cells in intermediate layers (III–V) of prelimbic cortex. Panel A3 shows a higher magnification of individual cells expressing both transcripts in the same area. Bottom row (B1–B3). $\alpha_{1B}$AR mRNA was also found in GABAergic cells. Panels B1 and B2 show the presence of $\alpha_{1B}$AR mRNA in some GABAergic cells of layers I and II–III, respectively, of the prelimbic cortex. Panel B3 shows a double-labelled cell in layers II–III of prelimbic cortex at a higher magnification. Red arrowheads indicate double-labelled cells. Blue arrowheads indicate cells only positive for $\alpha_{1B}$AR mRNA. Bar size, 20 $\mu$m.
two-way ANOVA) (Fig. 5). GABAergic neurons expressing \(\alpha_1\)D DAR were also localized in superficial layers (layer factor: \(F_{1,12} = 53.3, p < 0.0001\); region factor: \(F_{1,12} = 8.6, p < 0.05\); two-way ANOVA) (Fig. 5).

Co-expression of \(\alpha_1\)ARs and 5-HT\(_{2A}\)Rs in rat PFC

The regional distribution of \(\alpha_1\)AR and 5-HT\(_{2A}\)AR mRNA was very similar in PFC (Fig. 6). Figure 6b corresponds to the combined expression of all three \(\alpha_1\)AR subtypes, obtained by hybridizing PFC sections with a combination of the oligonucleotides selective for \(\alpha_{1A}\), \(\alpha_{1B}\) and \(\alpha_{1D}\) adrenergic receptor mRNA, as described in the Method section.

With the exception of layer VI, and the more ventral part of the infralimbic cortex, with low 5-HT\(_{2A}\)AR mRNA expression, virtually all other areas in PFC showed an abundant expression of both receptor transcripts. These areas included the cingulate and prelimbic areas of the mPFC, motor, agranular insular, orbital and piriform cortices, and TT. (See Fig. 1 for a detailed comparison of the expression of 5-HT\(_{2A}\)ARs with each one of the three \(\alpha_1\)AR subtypes.)

Double in situ hybridization experiments were conducted to examine the possible cellular co-localization of 5-HT\(_{2A}\)R with \(\alpha_{1A}\), \(\alpha_{1B}\) and \(\alpha_{1D}\) adrenergic receptors, as well as with the three subtypes together (total \(\alpha_1\)AR). Figures 6 and 7 show the extensive cellular co-expression of 5-HT\(_{2A}\)Rs with \(\alpha_1\)ARs, especially with \(\alpha_{1A}\)AR, and \(\alpha_{1D}\)AR subtypes, in various fields of the mPFC as well as in other PFC areas such as piriform cortex, TT or agranular insular cortex. We performed a detailed estimation of the percentage of cellular co-expression of 5-HT\(_{2A}\)Rs with each one of the three \(\alpha_1\)AR subtypes.)

Table 1. Expression of \(\alpha_1\) adrenergic receptors in pyramidal (vGluT1-positive) and GABAergic (GAD\(_{65/67}\)-positive) neurons of the rat mPFC

<table>
<thead>
<tr>
<th>Area</th>
<th>Layers II–III</th>
<th>Layer V</th>
<th>Layer VI</th>
</tr>
</thead>
<tbody>
<tr>
<td>vGluT1-positive</td>
<td>85 ± 4*</td>
<td>71 ± 9</td>
<td>65 ± 8</td>
</tr>
<tr>
<td>ACAd</td>
<td>80 ± 3*</td>
<td>79 ± 8*</td>
<td>59 ± 2</td>
</tr>
<tr>
<td>PL</td>
<td>62 ± 8</td>
<td>63 ± 8</td>
<td></td>
</tr>
<tr>
<td>ILA</td>
<td>72 ± 5*</td>
<td>57 ± 8</td>
<td></td>
</tr>
<tr>
<td>GAD-positive</td>
<td>79 ± 10</td>
<td>73 ± 14</td>
<td>62 ± 1</td>
</tr>
<tr>
<td>ACAd</td>
<td>79 ± 5*</td>
<td>72 ± 5</td>
<td></td>
</tr>
<tr>
<td>PL</td>
<td>74 ± 3</td>
<td>52 ± 6**</td>
<td></td>
</tr>
<tr>
<td>ILA</td>
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</tbody>
</table>

vGluT1, Vesicular glutamate transporter 1; GAD, glutamate decarboxylase; PFC, prefrontal cortex.

Data (mean ± S.E.M.) are percentages of pyramidal or GABAergic neurons expressing total \(\alpha_1\)ARs in the various cortical layers of the dorsal anterior cingulate (ACAd), prelimbic (PL) and infralimbic (ILA) areas of the rat mPFC. * \(p < 0.05\) vs. layer VI, ** \(p < 0.05\) vs. layers I–III, Tukey’s test post-ANOVA. Data are means ± S.E.M. of three rats (triplicate sections per rat).
Fig. 5. Bar graph showing the percentage of pyramidal (vesicular glutamate transporter 1-positive) and GABAergic (GAD-positive) neurons expressing each α1-adrenergic receptor subtype in superficial (II–III) and deep (V–VI) layers of infralimbic (ILA), prelimbic (PL) and cingulate (ACAd) subdivisions of medial prefrontal cortex. * p < 0.05 vs. deep layers of the same area, Newman–Keuls test post-ANOVA. Data are means ± S.E.M. of three rats (duplicate sections per rat).

Fig. 6. Low magnification dark-field photomicrographs showing the localization of (a) 5-HT2A mRNA, and (b) α1 adrenergic receptor mRNA in rat prefrontal cortex (PFC) using in situ hybridization histochemistry. The sections correspond approximately to AP +3.7 mm (Paxinos & Watson, 2005). Each receptor transcript was labelled with 33P-labelled oligonucleotides. (a) As previously described (Amargo’s-Bosch et al. 2004; Santana et al. 2004), cells expressing 5-HT2A Rs are located in most dorsal and ventral PFC regions, yet a low density of expressing cells was found in the infralimbic area and layer VIa of the mPFC. Note the presence of cells containing the 5-HT2A transcript in various cortical layers. This was particularly evident in dorsal and lateral aspects of PFC. (b) Cells expressing one or more type of α1 ARs were still more abundant than those expressing 5-HT2A Rs. Virtually all areas of the PFC showed the presence of α1A, α1B, and α1D adrenergic receptor mRNA. Open squares indicate the approximate location of areas where co-expression of both receptors was examined (Fig. 6). Bar size, 1 mm.
Fig. 7. Co-localization of 5-HT$_2A$ mRNA (Dig-labelled oligonucleotides, dark precipitates) with the different types of $\alpha_1$ adrenergic receptor (AR) mRNA ($^{33}$P-labelled oligonucleotides, silver grains) in various areas of the rat prefrontal cortex (PFC).

(A1–A3) Co-localization with $\alpha_{1A}$ARs in the prelimbic cortex; Panels A1 and A3 show, at different magnification, cells expressing both receptors mRNA in layers II–III whereas panel A2 shows some cells in layer VI. Note the presence of cells positive for $\alpha_{1A}$ARs and negative for 5-HT$_2A$Rs (blue arrowheads), as expected from the scarcity of cells expressing 5-HT$_2A$Rs and abundance of $\alpha_{1A}$ARs in deep layers. (B1–B3) Co-localization of 5-HT$_2A$Rs with $\alpha_{1B}$ARs in layers II–III of the anterior cingulate (B1 and B3) and prelimbic (B2) cortices. As expected from the negative dorsoventral gradient of expression of $\alpha_{1B}$ARs in the mPFC, there was a greater co-expression in cingulate cortex. (C1–C3) Co-localization of 5-HT$_2A$Rs with $\alpha_{1D}$ARs in layers II–III of the anterior cingulate (C1 and C3) and layer VIb of the prelimbic cortex (C2). Note the absence of the $\alpha_{1D}$AR transcript in deep layers, and consequently, the low level of co-expression with 5-HT$_2A$Rs. (D1–D3) Co-localization of 5-HT$_2A$R mRNA with $\alpha_{1A}$, $\alpha_{1B}$, and $\alpha_{1D}$ adrenergic receptor mRNA (multiple probes). Panels D1 and D3 correspond to layers II–III and panel D2 corresponds to layer VI, all in prelimbic cortex. Regarding individual $\alpha_1$AR mRNA, note the low co-expression in layer VI due to the small number of 5-HT$_2A$-positive cells in this area. Several cells positive for one or more $\alpha_1$AR mRNA (e.g. the $\alpha_{1A}$ subtype) and negative for 5-HT$_2A$R mRNA are indicated with blue arrowheads. Panels E1–E3 show the high co-expression of both receptors in areas other than the mPFC. Panel E1 shows the co-localization of the 5-HT$_2A$R mRNA with $\alpha_{1A}$AR mRNA in tenia tecta. Panels E2 and E3 show, respectively, the co-localization of 5-HT$_2A$R mRNA with $\alpha_1$AR mRNA (multiple probes) in the piriform cortex and the ventral part of the agranular insular cortex. Bar size, 20 $\mu$m.
percentage of cells also expressing α₁ARs varied between 44% in the infralimbic area to 75% and 80%, respectively, in the prelimbic and cingulate areas. Given the preferential expression of α₁ARs in superficial layers, where cell counts were performed, this subtype was probably the one contributing most to co-expression with 5-HT₁A Rs. However, it is likely that similar counts performed in areas showing abundant expression of 5-HT₁A Rs and α₁ARs, such as the motor area or the agranular insular cortex, would also give very high levels of co-expression, as shown in Fig. 6.

Discussion

The present study shows that α₁AR mRNA is expressed in rat PFC in a very high proportion of pyramidal neurons and local GABAergic interneurons. The three α₁AR subtypes show a complementary and almost non-overlapping distribution in the different PFC subdivisions and layers, which suggests specific roles for each α₁AR in the various PFC functions. Interestingly, cells expressing α₁ARs were significantly more abundant in deep layers whereas cells expressing α₂D ARs were much more abundant in superficial layers, suggesting that the former receptors are involved in the control of subcortical structures. Each α₁AR type was extensively co-expressed with 5-HT₁A Rs, which indicates a convergence of excitatory noradrenergic and serotonergic signals in the same PFC neurons.

To our knowledge, this is the first study examining the cellular localization of α₁AR mRNA in mammalian PFC. It adds to similar studies on 5-HT and dopamine receptors relevant for antipsychotic drug action (Amargós-Bosch et al. 2004; Puig et al. 2004; Santana et al. 2004, 2009). The three α₁AR types occur in various cortical areas (Day et al. 1997; Palacios et al. 1987; Pieribone et al. 1994). When examined in a study by Pieribone et al. (1994), their regional distribution coincides essentially with the present observations. The use of double in situ hybridization enabled us to quantify the proportion of pyramidal and GABAergic neurons expressing each α₁AR type. Interestingly, the proportion of each neuronal type expressing one or more types of α₁AR was surprisingly high (80–86%), compared to 5-HT₁A, 5-HT₁B, 5-HT₂, 5-HT₄ or dopamine D₁ and D₂ receptors in rat PFC using the same methodology (maximum of 66% for 5-HT₁A Rs in ACAd) (Puig et al. 2004; Santana et al. 2004, 2009; T. Vilaró, personal communication).

The difference between the proportion of PFC GABAergic interneurons expressing α₁ARs or other monoaminergic receptors is even more marked. Hence, a maximal proportion of 32–34% GABAergic neurons in ACAd/PL express 5-HT₁A Rs (Santana et al. 2004). Moreover, 5-HT₁A Rs, which are exclusively expressed by GABAergic interneurons in PFC and hippocampus (Morales et al. 1996; Puig et al. 2004) are present in 6–40% of GABAergic neurons in different cortical layers (Puig et al. 2004). Similarly, dopamine D₁ and D₂ receptor mRNA is present in 5–38% of GABAergic neurons in various PFC subdivisions (Santana et al. 2009). However, the different α₁ARs were expressed in a much higher proportion of GABAergic interneurons, similar to that in pyramidal neurons (69–74% in superficial layers; 52–73% in deep layers). Overall, the presence of α₁ARs in such a large proportion of pyramidal and GABAergic neurons suggests that NE can modulate the activity of most PFC output neurons via direct or indirect actions, a view consistent with electrophysiological observations, some of which are common with those of 5-HT₁A activation (Araneda & Andrade, 1991; Marek & Aghajanian, 1999). Moreover, the presence of 5-HT₁A Rs and α₁ARs in GABAergic interneurons indicates that 5-HT and NE may play a prominent role in the control of local PFC networks, as observed in electrophysiological experiments. Hence, both NE and 5-HT can inhibit the activity of layer V pyramidal cells via activation of 5-HT₁A Rs and α₁ARs in fast-spiking GABAergic cells (Kawaguchi & Shindou, 1998; Puig et al. 2010; Weber & Andrade, 2010; Zhong & Yan, 2011).

The NE-evoked excitations of pyramidal neurons involve post-synaptic α₁ARs. However, there have been controversial views on the localization of 5-HT₁A Rs in...
PFC. Hence, while some studies suggested a presynaptic localization on thalamocortical afferents (Aghajanian & Marek, 1997, 1999), subsequent studies did not support this view (Beique et al. 2007; Miner et al. 2003; Puig et al. 2003), indicating a predominant post-synaptic localization of 5-HT₁A Rs in PFC neurons, in common with α₁ARs. A recent paper showed the presence of α₁AR immunoreactivity in vGlut1-positive glutamatergic axons in PFC (Mitrano et al. 2012). This would be consistent with the presence of a large proportion of layer III neurons expressing α₁AR transcripts (present study), suggesting that NE may also modulate intracortical connectivity through presynaptic excitatory α₁ARs.

The markedly high co-expression of 5-HT₂A R mRNA with that of α₁ARs suggests a convergence of excitatory signals evoked by 5-HT and NE on PFC neurons. Given the different proportion of GABA cells expressing 5-HT₁A Rs and α₁ARs, it is likely that most of the co-expression takes place in pyramidal neurons. Indeed, immunohistochemical studies have shown that α₁ARs and 5-HT₂A Rs are localized in cell bodies and apical dendrites of pyramidal neurons (Acosta-Martínez et al. 1999; Cornea-Hebert et al. 1999; Jakab & Goldman-Rakic, 1998, 2000; Martin-Ruiz et al. 2001). Further, functional interactions have been reported between 5-HT₁A Rs and α₁ARs in the rat (see Introduction). These observations, together with the common signalling pathways and their functional interactions, suggest a close association between both receptors. Whether this involves the formation of stable heteromers, as observed with other neurotransmitter receptors, remains to be established. Preliminary data indicate that 5-HT₁A Rs co-immunoprecipitate and show stable fluorescence resonance energy transfer (FRET) signals with all three α₁ARs expressed in HEK-293T cells (Santana et al. unpublished observations). However, this is just a first step in establishing the formation of receptor heteromers in vivo (Ferré et al. 2009).

The present observations may have important physiological and therapeutic implications. On the one hand, PFC neuronal activity may be concurrently regulated by the ascending 5-HT and noradrenergic systems. Since both neuronal groups are REM-off (Aston-Jones et al. 1991; Jacobs & Azmitia, 1992) the concurrent activation (or its lack) of excitatory α₁ARs and 5-HT₁A Rs in the same cells may have a synergistic effect on the activity of cortical neurons during wakefulness and sleep. Moreover, both receptors have been involved in working memory through the modulation of neuronal activity in dorsolateral PFC (Ramos & Arnsten, 2007; Williams et al. 2002). On the other hand, dual reuptake inhibitors (Artigas, 1995) show an antidepressant efficacy higher than selective 5-HT reuptake inhibitors, an effect which may occur via summation of effects on the same cortical neurons. Finally, conventional and second-generation (atypical) antipsychotics share the ability to antagonize α₁ARs while the latter drugs also antagonize 5-HT₁A Rs (Arnt & Skarsfeldt, 1998; Bymaster et al. 1996). The therapeutic potential of α₁AR blockade in schizophrenia has been neglected due to its cardiovascular effects, despite the fact that prazosin enhanced schizophrenia and serotonin(2A) receptor blockade (Wadenberg et al. 2000). The present observations support the view that α₁AR and 5-HT₁A R blockade may add to each other to modulate PFC function making the PFC a particularly sensitive area to the action of atypical antipsychotic drugs (Artigas, 2010).

In summary, the present study shows that the ascending 5-HT and NE systems share common actions in the PFC through the concurrent expression of α₁ARs and 5-HT₁A Rs in most PFC neurons, an observation with potential physiological and pharmacological consequences.

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Statement of Interest

None.

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