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Short communication

α_{2A}-adrenoceptors enhance the serotonergic effects of fluoxetine

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Abstract

The ability of subtype-preferring α_2-adrenoceptor antagonists to enhance the neurochemical effects of the antidepressant, fluoxetine, was evaluated by in vivo microdialysis. Combining the selective α_{2A}-adrenoceptor antagonist, BRL-44408 (10 mg/kg, s.c.), with fluoxetine (30 mg/kg, s.c.) elevated the extracellullar levels of serotonin (5-HT) and noradrenaline in the rat frontal cortex, an effect not observed following antidepressant treatment alone. In contrast, combining fluoxetine with the α_{2B}- or α_{2C}-adrenoceptor antagonists, imiloxan (10 mg/kg, s.c.) or rauwolscine (10 mg/kg, s.c.), respectively, did not similarly alter biogenic amine levels. Collectively, these results reveal a specific role for the α_{2A}-adrenoceptor subtype in augmenting the neurochemical effects of antidepressants.

Keywords: Depression; Adrenergic receptor; SSRI (Selective Serotonin Reuptake Inhibitor); Microdialysis

1. Introduction

A limitation of all marketed antidepressants is that approximately one-third of patients are unresponsive to their treatment. Furthermore, in patients that do respond, there is typically a 3 to 6 week delay in the onset of antidepressant activity. One strategy to circumvent these issues is to combine traditional antidepressants like the selective serotonin reuptake inhibitors (SSRIs) with non-selective α_2-adrenoceptor antagonists such as mirtazapine or yohimbine (reviewed in Schechter et al. (2005)). The support for this approach is endorsed by recent clinical findings showing that such combination therapies help with treatment-resistant depression and can actually shorten the time required to achieve antidepressant activity (Sanacora et al., 2004; Aydemir et al., 2005).

Understanding the adrenoceptor subtype responsible for the neurochemical effects of combining SSRIs with α_2-adrenoceptor antagonists should facilitate the discovery of novel antidepressants. Presumably, such agents would possess antidepressant activity and, by virtue of their ability to influence both 5-HT and noradrenaline neurotransmission, be effective in treatment-resistant depression and/or exhibit an accelerated onset of activity. To determine which of the 3 known α_2-adrenoceptor subtypes, α_{2A}, α_{2B}, and α_{2C}, influence the augmentation of antidepressants, in vivo microdialysis techniques in rats were performed to evaluate the neurochemical effects of combining subtype-preferring α_2-adrenoceptor antagonists with the SSRI, fluoxetine.

2. Materials and methods

2.1. Animals and in vivo microdialysis surgery

All in vivo studies were conducted according to the specifications of both the National Institutes of Health guide for the Care and Use of Laboratory Animals (Pub. 85-23, 1985) and Wyeth's Institutional Animal Care and Use Committee. Using 2–3% halothane anesthesia (Fluothane; Zeneca, Cheshire, UK), male Sprague–Dawley rats (280–350 g; Charles River, Wilmington, MA) were secured in a stereotaxic frame with ear and incisor bars (David Kopf, Tujunga, CA) while a microdialysis guide cannula (CMA/12, CMA Microdialysis, Sweden) was directed above the dorsal lateral frontal cortex (A/P +3.2 mm, M/L −3.5 mm, D/L −1.3 mm). Coordinates were taken with a flat skull using the rat brain atlas of Paxinos and Watson (1986). Also at this time, a subcutaneous cannula (s.c.) was implanted between the shoulder blades of the animals. Both the guide and

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s.c. cannulae were secured to the skull with two stainless-steel screws (Small Parts, Roanoke, VA) and dental acrylic (Plastics One, Roanoke, VA). Following a 24 h post-operative recovery, the animals were individually housed in Plexiglas cages (45 cm sq.) where they had free access to water and standard rat chow.

2.2. In vivo microdialysis procedures

For in vivo neurochemical experiments, a microdialysis probe (CMA/12; active membrane length 2 mm; OD 0.5 mm; 20 kDa cut-off) was pre-washed with artificial CSF (aCSF; 125 mM NaCl, 3 mM KCl, 0.75 mM $MgSO_4$ and 1.2 mM $CaCl_2$, pH 7.4) according to the manufacturer's specifications. In the morning of the study, probes were inserted, via the guide cannula, into the frontal cortex and perfused with aCSF at 1 μl/min. Following a 3 h stabilization period, four control samples (20 μl) were collected to establish a steady baseline. Immediately following the last baseline sample, the α_{2A}, α_{2B}, or α_{2C}-adrenoceptor antagonists, BRL-44408 (10 mg/kg, s.c.), imiloxan (10 mg/kg, s.c.) or rauwolscine (10 mg/kg, s.c.), respectively, were injected 20 min before fluoxetine (30 mg/kg, s.c.). Subsequent microdialysis samples were collected for 3 h post-injection and analysed for 5-HT and noradrenaline content by high performance liquid chromatography (HPLC).

2.3. Analysis of 5-HT and noradrenaline

The HPLC conditions have been described previously (Beyer et al., 2002). Briefly, a mobile phase (0.15 M NaH_2PO_4, 0.25 mM EDTA, 1.75 mM 1-octane sulphonic acid, 2% isopropanol and 4% methanol, pH=4.6) was delivered by a Jasco PU1580 HPLC pump (Jasco Ltd, Essex, U.K) at a flow rate of 0.5 μl/min. Separation was achieved with a reverse phase column (C18 ODS3 column, 150×3.0 mm, Varian Inc, CA) and an ANTEC electrochemical detector (ANTEC, Netherlands) was used to quantify the levels of 5-HT and noradrenaline. The detector was set at a potential of 0.65 V vs. an Ag/AgCl reference electrode. All neurochemical data were acquired using the Atlas software package (Thermo Labsystems, Beverley, MA) and compared to an external standard curve. The fmol concentrations of all neurotransmitters during the baseline samples were averaged and this value was denoted as 100%. Subsequent sample values were expressed as a percent change from this preinjection baseline value (or % change from baseline). Neurochemical data, excluding preinjection values, were analysed by a two-way analysis of variance (ANOVA) with repeated measures (time). Post hoc analyses were made using the Bonferroni/Dunns adjustment for multiple comparisons.

2.4. Chemical compounds and reagents

All drugs including BRL-44408 maleate (2-[2*H*-(1-methyl-1,3-dihydroisoindole) methyl]-4,5-dihydroimidazole), imiloxan HCl (±)-2-(1-ethyl-2-imidazoyl)methyl-1,4-benzodioxane hydrochloride, rauwolscine HCl (17a-Hydroxy-20a-yohimban-16b-carboxylic acid, methyl ester hydrochloride), and fluoxetine HCl (*N*-methyl-3-phenyl-3-[4-(trifluoromethyl)phenoxy]-propan-1-amine) were purchased from Tocris (Ellisville, MO). All HPLC chemicals were of analytical grade and purchased from Sigma-Aldrich chemicals (Milwaukee, WI).

3. Results

3.1. α_{2A}-adrenoceptors enhance the serotonergic effects of fluoxetine

Similar to previous results in rats (Dawson et al., 2000; Beyer et al., 2002), acute fluoxetine (30 mg/kg, s.c.) administration did not significantly alter the frontal cortex levels of either 5-HT ($P=0.1603$) or noradrenaline ($P=0.3269$; Fig. 1A). Combining the selective α_{2A}-adrenoceptor antagonist BRL-44408 (10 mg/kg, s.c.) with fluoxetine elicited a maximal 80% increase in 5-HT, which is similar in magnitude (~ 2-fold) and duration to that

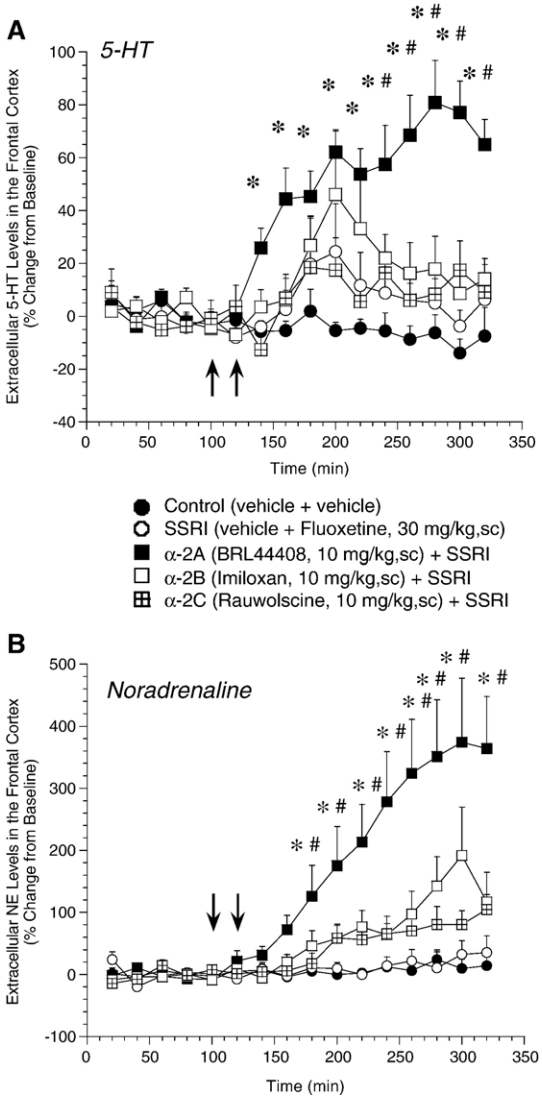

Fig. 1. α_{2A}, but not α_{2B} or α_{2C}, adrenoceptor antagonism enhance the serotonergic (panel A) and noradrenergic (panel B) effects of fluoxetine. Data are expressed as mean±S.E.M. $N=8$–12 rats per group. Arrows designate the subcutaneous drug injection. *$P<0.05$ compared to vehicle-treated animals. $^{\#}P<0.05$ compared to α_{2B} or α_{2C} antagonism.

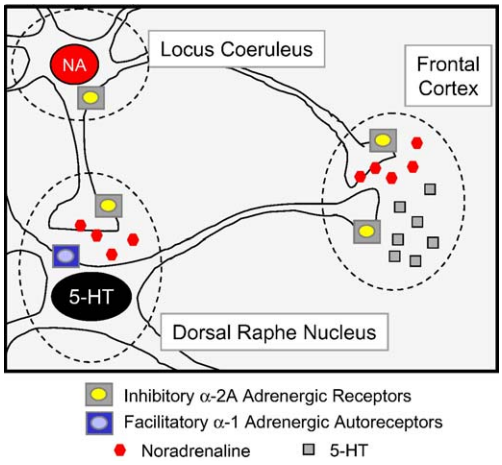

Fig. 2. Proposed blockade of α_{2A}-adrenoceptors autoreceptors in the frontal cortex and dorsal raphe elevates the local levels of noradrenaline. Increases in cortical 5-HT likely occur by combining 5-HT transporter blockade with the antagonism of either α_{2A}-adrenoceptors located on serotonergic terminals and/or increased dorsal raphe noradrenaline levels that engage local stimulatory α_1-adrenoceptors to stimulate the 5-HT release in cortical areas.

seen following chronic, 14-day fluoxetine treatment (Dawson et al., 2000). A robust 374% elevation in cortical noradrenaline levels was also observed in these same animals after administration of the SSRI/α_{2A}-adreneoceptor combination (Fig. 1B). The magnitude of this response is consistent with previous data (Pudovkina et al., 2001) showing that BRL-44408 alone sufficiently increases the extracellular noradrenaline implying that the noradrenergic results of the present study are likely due exclusively to the antagonism of α_{2A}-adrenergic autoreceptors. A one-way ANOVA with repeated measures indicated a significant treatment [5-HT: $F(1,18)=35.07$, $P<0.0001$; noradrenaline: $F(1,15)=8.07$, $P<0.0124$] and interaction [5-HT: $F(9,162)=3.27$, $P=0.0011$; noradrenaline: $F(9,135)=5.91$, $P<0.0001$] effect when α_{2A}-adrenoceptor antagonism was combined with this SSRI. In contrast, both the α_{2B}- and α_{2C}-preferring adrenoceptor antagonists, imiloxan (10 mg/kg, s.c.) and rauwolscine, failed to evoke similar changes in monoamines when combined with fluoxetine (Fig. 1A and B). Maximal neurochemical changes following α_{2B} and α_{2C} antagonism were as follows: imiloxan, 5-HT: 46%, noradrenaline: 191%; rauwolscine, 5-HT: 18%, noradrenaline: 104%.

4. Discussion

These microdialysis results indicate that the subtype selective antagonism of α_{2A}-adrenoceptors markedly augments the serotonergic effects of fluoxetine. Although these data do not completely eliminate the possibility that related α_2-adrenoceptors contribute to the potentiation of the effects of fluoxetine, they are entirely consistent with data from α_{2A}-adrenoceptor knockout mice showing that this receptor subtype is the predominant adrenergic autoreceptor mediating the release of biogenic amines (Lähdesmäki et al., 2003). Moreover, these data corroborate preclinical reports that non-selective α_2-adrenoceptor antagonists such as atipamezole and idazoxan enhance the effects of various antidepressants on noradrenergic and serotonergic neurotransmission (Gobert et al., 1997; Dawson et al., 1999).

The ability of α_2-adrenoceptors to serve as inhibitory autoreceptors and exert pronounced effects on 5-HT and noradrenaline levels is well characterized. However, the present studies are the first to reveal a specific role for the α_{2A}-adrenoceptor subtype in augmenting the neurochemical effects of an SSRI. The proposed circuitry for how α_{2A}-adrenoceptors influence these responses is illustrated in Fig. 2. By blocking the inhibitory α_{2A} autoreceptors located on noradrenergic nerve terminals in both the frontal cortex and dorsal raphe nuclei, BRL-44408 elicits pronounced elevations in local noradrenaline (Pudovkina et al., 2001, 2003). Consistent with these reports, the present study found that combining BRL-44408 with fluoxetine produced marked elevations in noradrenaline. Increases in 5-HT likely occur by combining the 5-HT transporter blockade with the antagonism of either α_{2A}-adrenoceptors located on serotonergic terminals and/or increased dorsal raphe noradrenaline levels that engage the local stimulatory α_1-adrenoceptors to stimulate 5-HT release in cortical areas (see Discussion section in Gobert et al. (1997) and Dawson et al. (1999)).

The results of the present study are the first to show that the selective subtype antagonism of the α_{2A}-adrenoceptor markedly influences the neurochemical effects of fluoxetine. These collective data have clinical implications in that they suggest combining 5-HT reuptake inhibition with α_{2A}-adrenoceptor antagonism – either in a single molecule or as an adjunctive therapy – could have a therapeutic utility in the treatment of depression. Furthermore, based on recent clinical findings (Sanacora et al., 2004; Aydemir et al., 2005), such combination approaches would be expected to yield effective antidepressant activity with an accelerated onset of action.

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