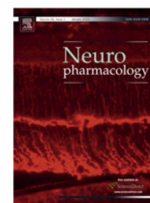
Neuropharmacology 62 (2012) 2354–2362

Contents lists available at SciVerse ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm



Noradrenergic antidepressant responses to desipramine *in vivo* are reciprocally regulated by arrestin3 and spinophilin

Christopher Cottingham [a], Xiaohua Li [b,1], Qin Wang [a,*]

[a] Department of Physiology & Biophysics, University of Alabama at Birmingham, 986 MCLM 1918 University Blvd., Birmingham, AL 35294, USA
[b] Department of Psychiatry & Behavioral Neurobiology, University of Alabama at Birmingham, Birmingham, AL 35294, USA

A R T I C L E I N F O

Article history:
Received 12 October 2011
Received in revised form
9 February 2012
Accepted 12 February 2012

Keywords:
α_2 Adrenergic receptor
Arrestin
Depression
Desipramine
Forced swim test
Spinophilin

A B S T R A C T

Many antidepressant drugs, including the tricyclic antidepressant desipramine (DMI), are broadly understood to function by modulating central noradrenergic neurotransmission. α_2 adrenergic receptors (α_2ARs) are key regulators of the noradrenergic system, and previous work has implicated α_2ARs in mediating the antidepressant activity of DMI in the rodent forced swim test (FST). However, little is known about intracellular regulators of antidepressant drug action. α_2AR function is tightly regulated by its intracellular interacting partners arrestin and the dendritic protein spinophilin. We have previously established the competitive and reciprocal nature of these interacting proteins at the α_2AR in the context of classic agonist effects, and have shown DMI to be a direct arrestin-biased ligand at the receptor. In the present study, we report that mice deficient in the α_{2A}AR subtype lack DMI-induced antidepressant behavioral effects in the FST. As well, mice deficient in arrestin3 lack antidepressant response to DMI, while spinophilin-null mice have enhanced antidepressant response to DMI compared with wild-type controls, indicating that this α_{2A}AR-mediated response is reciprocally regulated by arrestin and spinophilin. The characteristic of α_{2A}AR-dependence and arrestin3 involvement was shared by the antidepressant effect of the classic α_2AR agonist clonidine but not the non-tricyclic norepinephrine reuptake inhibitor reboxetine, supporting a model whereby DMI exerts its antidepressant effect through direct engagement of the α_{2A}AR and arrestin3. Our results implicate arrestin- and spinophilin-mediated regulation of the α_{2A}AR in the pharmacology of the noradrenergic antidepressant DMI, and suggest that manipulation of this mode of receptor regulation may represent a novel and viable therapeutic strategy.
© 2012 Elsevier Ltd. All rights reserved.

1. Introduction

Current therapeutic strategies in depressive disorders rely almost entirely on modulation of monoamine neurotransmitter systems in the brain, with a large proportion of antidepressants functioning as reuptake inhibitors for the monoamine norepinephrine (NE). This reuptake inhibition occurs via blockade of the NE transporter (NET) leading to some modulation of noradrenergic neurotransmission. While this much is known, a complete understanding of the noradrenergic antidepressant mechanism of action remains elusive.

In addition to NET, other key players in the noradrenergic system include the α_2 adrenergic receptors (ARs), in particular the α_{2A}AR subtype, which is the predominant subtype expressed in the central nervous system (De et al., 1992; Sastre and Garcia-Sevilla, 1994; Wang et al., 1996). The α_{2A}AR is a key regulator of the noradrenergic system, with important functions as a presynaptic autoreceptor controlling synthesis and release of NE, a postsynaptic heteroreceptor regulating responses to NE, and a presynaptic heteroreceptor regulating release of other neurotransmitters (Hein et al., 1999; Knaus et al., 2007; Gilsbach et al., 2009; Shields et al., 2009). α_{2A}AR function is itself tightly regulated by interacting protein partners. The non-visual arrestins (arrestin2 & 3, also called β-arrestin1 & 2) are key regulators of GPCR function, responsible for attenuation of receptor signaling and mediating endocytosis of receptors via clathrin-coated pits (Shenoy and Lefkowitz, 2003). Further, G protein-independent arrestin-mediated signaling responses are becoming increasingly appreciated at GPCRs (Violin and Lefkowitz, 2007; Rajagopal et al., 2010). As well, we have previously established the dendritic scaffolding protein spinophilin

Abbreviations: 5HT, serotonin; AR, adrenergic receptor; Arr, arrestin; DMI, desipramine; EPM, elevated plus maze; FLX, fluoxetine; FST, forced swim test; GPCR, G protein-coupled receptor; NET, norepinephrine transporter; Sp, spinophilin; TST, tail suspension test.

* Corresponding author. Tel.: +1 205 996 5099; fax: +1 205 975 9028.
E-mail address: qinwang@uab.edu (Q. Wang).
[1] Present address: St. Vincent's Health System, St. Vincent's East, Birmingham, AL 35209, USA.

0028-3908/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuropharm.2012.02.011

(Allen et al., 1997; Satoh et al., 1998) as a functional antagonist of arrestin at the α_{2A}AR (Wang et al., 2004). The interactions of arrestin and spinophilin with the α_{2A}AR are competitive in nature, and loss of either protein has opposing effects on the *in vivo* α_2AR agonist-induced α_{2A}AR-mediated sedation response (Wang et al., 2004).

The classic tricyclic antidepressant desipramine (DMI) is a potent and selective blocker of NET (Baldessarini, 2006) thought to function primarily through modulation of noradrenergic transmission, modulation which results in antidepressant effects on behavior. As well, we have recently reported that DMI, acting as a direct α_{2A}AR ligand, drives recruitment of arrestin to the receptor thereby modulating receptor function (Cottingham et al., 2011). Importantly, α_2ARs have been implicated in mediating the antidepressant activity of DMI, given that α_2AR antagonist treatment blocks its antidepressant effects in rodent models (Cervo et al., 1990; Reneric et al., 2001; Zhang et al., 2009). These findings indicate that α_2AR activation is required for the antidepressant behavioral effects of DMI in rodents. The antidepressant efficacy of α_2AR activation is also supported by the finding that the α_2AR agonist clonidine elicits an antidepressant behavioral response (Cervo and Samanin, 1991).

Although arrestin3-null mice are known to have altered behavioral responses to pharmacological manipulations (Schmid and Bohn, 2009) and we have shown that spinophilin-null mice have a range of enhanced responses to α_2AR agonists *in vivo* (Lu et al., 2010), neither of these models has been used to investigate potential roles for these proteins in the antidepressant behavioral response. In particular, whether reciprocal interplay between arrestin and spinophilin applies to the α_2AR-mediated antidepressant response to DMI is entirely unknown. Our previous findings do in fact strongly suggest roles for these proteins, given that DMI was shown to be an arrestin-biased ligand at the α_{2A}AR (Cottingham et al., 2011) and spinophilin has been established as an endogenous antagonist of arrestin (Wang et al., 2004).

In the present study, we set out to determine the specific role for the α_{2A}AR subtype in mediating the antidepressant response to DMI, and to investigate whether the interacting partners (arrestin and spinophilin) known to regulate α_{2A}AR function have a role in this response. We chose to model the antidepressant response using the Porsolt's forced swim test (FST) (Porsolt et al., 1977a,b), a sensitive and widely accepted model for assessing antidepressant drug activity in rodents. We hypothesized that DMI exerts an antidepressant effect on behavior in the FST in an α_{2A}AR-dependent fashion, and that this antidepressant response is reciprocally regulated by arrestin and spinophilin. The following study tests that hypothesis through the use of transgenic mouse models lacking α_{2A}ARs, arrestin3, and spinophilin, primarily in the FST, assaying for responses to acute DMI administration. Our data indicate that the mode of reciprocal regulation by arrestin and spinophilin can be expanded beyond α_2AR agonist pharmacology and into the neuropharmacology of antidepressants.

2. Materials and methods

2.1. Animals

All animals were housed in the AAALAC-accredited Animal Resources Program (ARP) facility at the University of Alabama at Birmingham in accordance with procedures of the Animal Welfare Act and the 1989 amendments to the Act, and all studies followed protocols approved by the UAB Institutional Animal Care and Use Committee. The ARP provides full animal care services and viral-free barrier facilities. Animals had free access to food (standard chow diet) and water, and were maintained on a 12-hour light/dark cycle. Animals were transported from the animal housing facility to the testing site building 24 hours prior to any behavioral testing. To avoid testing order effects, separate cohorts of animals were used for the behavioral tests. All efforts were made to minimize animal suffering and to reduce the number of animals used in this study.

Male mice of age 3–6 months were used for all studies. Wild-type (WT) control animals were C57BL/6 mice, either obtained from in-house breeding or purchased from Charles River Laboratories. The generation of α_{2A}AR-null (α_{2A}AR$^{-/-}$) mice (Altman et al., 1999), arrestin3-null (Arr3$^{-/-}$) mice (Bohn et al., 1999), and spinophilin-null (Sp$^{-/-}$) mice (Feng et al., 2000) has been previously described. All transgenic animals (α_{2A}AR$^{-/-}$, Arr3$^{-/-}$, and Sp$^{-/-}$) were backcrossed more than 10 generations to and maintained on C57BL/6 genetic background. Consistency in genetic background is vital, as it is well-established that different mouse strains have widely variable baseline phenotypes and responses to pharmacological manipulations in the FST (Petit-Demouliere et al., 2005; Jacobson and Cryan, 2007). Experimental animals were generated by interbreeding of homozygous animals. Pups were weaned at 3–4 weeks of age, and housed in groups of 4–5 males per cage until use for testing. Sp$^{-/-}$ animals were housed in smaller groups (2–3) due to aggression and infighting between males in this mouse line.

2.2. Open field and elevated plus maze (EPM) tests

Data was acquired using the automated EthoVision camera-driven tracker system (Noldus, The Netherlands), the procedures for which have been previously described (Polter et al., 2009). The manufacturer's software was used to extract the relevant parameters from the raw data. Open field trials were conducted in an open field arena of area 42 cm^2 and were 10 min in length, with total distance traveled and relative time spent in the center of the open field used as endpoints. EPM test was carried out 24 hours following open field, with trials 4 min in length; data consisted of entries into and time spent in open versus closed arms of the EPM. Entries into each arm type were expressed as a percentage of total arm entries, and time spent in each arm type was calculated as a percentage of the total time after first closed arm entry. This calculation normalizes for variation which exists in animals' latency to first closed arm entry.

2.3. Drugs

Desipramine hydrochloride (DMI) and clonidine hydrochloride were obtained from Sigma. Reboxetine mesylate was obtained from Tocris Bioscience. Fluoxetine hydrochloride was generously provided by the NIMH Chemical Synthesis and Drug Supply Program. For animal treatments, drugs were prepared in vehicle (saline), with distilled water (less than 10% of final total volume) used to solubilize DMI, diluted to the appropriate concentration such that the injection volume was 10 ml/kg (e.g. DMI was prepared at a stock concentration of 2 mg/ml for a dose of 20 mg/kg). Drugs (or vehicle alone) were administered acutely via intraperitoneal (i.p.) injection.

2.4. Forced swim test (FST)

In our study, the FST was conducted using the automated Hamilton–Kinder device (Hamilton–Kinder, Poway, CA), controlled by the manufacturer's MotorMonitor software, as has been previously described (Kurtuncu et al., 2005; Polter et al., 2009). Tanks were equipped with two photobeam arrays allowing the animals' movements to be monitored and filled with 1 L of room temperature water (water changed between each trial). Trials were 6 min in length, with the last 4 min only counted for data acquisition. The MotorMonitor software was used to extract two primary parameters measuring the antidepressant response from the raw data: rest time (threshold set at 2 s), corresponding to time spent immobile; and basic movements (measured by photobeam breaks), corresponding to active swimming behaviors. Animals were tested 30 min following i.p. injection.

2.5. Tail suspension test (TST)

TST was conducted as previously described (Crowley et al., 2004; Polter et al., 2009), using an automated system (Med Associates Inc., St. Albans, VT). Output for the TST was time spent immobile. As in the FST, trials were 6 min in length, with the last 4 min only counted for data acquisition, and animals were tested 30 minutes following i.p. injection.

2.6. Statistics

Data were analyzed using GraphPad Prism software (GraphPad, San Diego, CA). Unpaired, two-tailed Student's *t*-tests were used to compare individual experimental groups with each other. Two-way ANOVA was used to analyze multiple experimental groups and test for drug × genotype interactions.

3. Results

3.1. Basic behavioral assessments

We began by performing basic behavioral assessments in our knockout mouse lines (in comparison with WT controls) to determine baseline activity (open field test) and anxiety (EPM test) levels. These assays provide valuable information about the inherent behavioral phenotypes of these animals, information

Download English Version:

https://daneshyari.com/en/article/2493526

Download Persian Version:

https://daneshyari.com/article/2493526

Daneshyari.com