Long-term efficacy of antidepressants: analyzing brain adaptive modifications.

Rebeca Vidal, Fuencisla Pilar-Cuellar, Antonio Rodriguez-Gaztelumendi, Jesús Pascual, Mª Luisa Rojo, Elena Castro, Alvaro Díaz, Elsa M. Valdizán and Angel Pazos*

Department of Physiology and Pharmacology, Faculty of Medicine, University of Cantabria, 39011, Santander, Cantabria, Spain. CIBER de Salud Mental (CIBERSAM), Instituto de Salud Carlos III, Spain

***Corresponding author:** Dr Angel Pazos, Department of Physiology and Pharmacology. University of Cantabria, Avda. Cardenal Herrera Oria s/n, 39011 Santander, Cantabria, Spain. Telephone: (34) 942201985. Fax number: (34)942201903. Email: *pazosa@unican.es*

Running title: Antidepressants-induced neural changes

Summary

The regulatory changes induced by chronic antidepressants on the different brain signalling process has been the subject of study in our group. We here review some of the results on this topic. On one side, our efforts have been addressed to the study of the coupling of 5-HT_1 receptors to G proteins: we have demonstrated that 5-HT_{1A} autoreceptors are selectively desensitized by chronic fluoxetine, suggesting that this could be one of the reasons of the delayed response of antidepressants. A functional desensitization of 5-HT_{1B} receptors has been also found. On the other hand, we have focused on the mechanisms involved in neural proliferation, studying 2 possible new targets: a) the endocannabinoid system, as we have observed a functional up-regulation of CB_1 receptor functionality in an animal model of depression (olfactory bulbectomy), reversed by fluoxetine; and b) the Wnt-β-catenin pathway: an up-regulation of the expression of β-catenin in hippocampus, in parallel with an increase of cell proliferation, has been observed in the hippocampus of rats treated with venlafaxine. Taken together, these results provide valuable information about the involvement of transductional pathways in the mediation of the effects of antidepressant drugs.

Depressive disorders are debilitating diseases with a high life prevalence[1.] The molecular mechanisms underlying the therapeutic action of antidepressant drugs (ADs) are not fully clarified: those most commonly used present as an immediate mechanism of action their ability to increase serotonin (5-HT) and/or norepinephrine (NE) brain levels. Since the initial introduction of tricyclic compounds, several pharmacological groups have been progressively incorporated to the therapy of depressive disorders: in this regard, selective inhibitors of 5-HT reuptake (SSRI) have represented a relevant landmark in the field. Dual NE and 5-HT reuptake inhibitors (SNRI) are a new alternative, still in the frame of monoaminergic acting drugs[2]. A huge number of compounds, exploring other non-monoaminergic mechanisms[3], are currently in development, although still without a clinical demonstration of efficacy.

Although the increase in monoamine levels is a short-term response of ADs, all these drugs need to be administered for at least 2-4 weeks to produce a significant clinical improvement. This lag is considered to be necessary for brain adaptive processes to occur[4-6]. It has been classically suggested that these long-term processes could be related to progressive changes in aminergic neurotransmission. In the recent years, other non-exclusive neurobiological theories propose that the functional efficacy of ADs could involve modifications in various signaling pathways regulating cellular plasticity and survival, leading to trophic responses[7].

The evidence that existing ADs treatments exhibit a limited efficacy and a slow onset of action suggests that this therapy has not yet reached their upper limit. Therefore, further research on new targets, in addition to increase our knowledge about the mechanisms underlying the antidepressant effect, will likely result in the discovery of drugs with higher profile of response and faster onset of action. In the last decade, our

group has been interested in the analysis of the intracellular mechanisms that are modified by chronic antidepressant treatments. This research has been mainly carried out in normal animals, but some studies in animal models of depression as well as in postmortem brain samples of depressed patients have also been carried out. In the following we will review some of this work.

Methods

In the studies reviewed below, we have used a number of experimental procedures (radiometric labeling, western blot, immunohistochemistry, enzyme quantiation, electrophysiology, behavioural approaches) in order to analyze in deep the involvement of intracellular mechanisms in the long-term response to antidepressants. We will not describe here in detail the different methodological approaches, that are fully reported in the original articles presenting these results [8-11].

Adaptive changes of monoaminergic systems: the case of 5-HT_1 receptors

5-HT-mediated neurotransmission is still one of the main identified targets for antidepressant action. Chronic administration of ADs results in regulatory changes of the different 5-HT receptor subtypes, which could be of relevance for the clinical response. 5-HT exerts its actions through at least 14 different receptor subtypes, the 5-HT_1 family, associated to G proteins, is present in high densities through the central nervous system. In this regard, our group has devoted considerable effort to analyze the modifications induced by ADs on the transductional mechanisms associated to the two main 5-HT_1 receptor subtypes: 5-HT_{1A} and 5-HT_{1B}. Indeed, the efficacy in coupling to G proteins has been one of the subjects of our study[8]. As it is illustrated in Figure 1A, chronic treatment with the SSRI fluoxetine (10 mg/kg, 21 days) induces a differential

response in the level of stimulation of [^{35}S]GTPγS binding, depending on the rat brain area analyzed: a significant desensitization is observed in the dorsal raphe, while non-significant changes occur in the remaining areas examined (i.e. hippocampus). 5-HT_{1A} receptors over the dorsal raphe are presynaptic and act as autoreceptors controlling the neuronal discharge. A desensitization of 5-HT_{1A} autoreceptors following AD treatment has been also found in vivo studies, including electrophysiological recordings[8,12-13].Our results demonstrate that this fluoxetine-induced desensitization of 5-HT_{1A} autoreceptors occurs at the G protein level. This finding is of special interest taking into account that it has been repeatedly suggested that this desensitization may be critical for the delayed onset of the antidepressant effect of SSRI[5]. With respect to 5-HT_{1B} receptors (Figure 1B), our studies reveal a general response of decrease in their G protein coupling ability throughout the rat brain (caudate-putamen, substantia nigra). Recent studies of our group show that this tendency to the decrease in 5-HT_{1B}-dependent functionality is also present in an animal model of depression (olfactory bulbectomy) in some (substantia nigra, -22.7%; dorsal raphe, -31.0%, $p<0.05$) but not all (caudate-putamen) areas (unpublished). The tendency to the functional desensitization of 5-HT_1 receptors can be explained in terms of regulatory response to the increase in the levels of synaptic 5-HT[14], due to the acute inhibition of the reuptake process. Although an exact correlation between these changes and the degree of efficacy of ADs is difficult to establish, it is tempting to speculate that these adaptive modifications in 5-HT-mediated signal transduction are required for the clinical response of these drugs.

Is there a role for the endocannabinoid system in the treatment of depression ? : the olfactory bulbectomy as a model.

Bilateral olfactory bulbectomy (OBX) in the rat is widely used as an animal model of depression, as these animals exhibit a number of behavioral, neurochemical and structural changes that are reversed by chronic ADs administration[15]. As current data suggest that brain endocannabinoid (EC) signalling, mainly through CB1 receptors, might be involved in the long-term adaptations induced by ADs, we have used this model to clarify this issue[11]. As shown in fig. 2A, an increased CB_1 receptor –mediated [^{35}S]GTP□S binding in the prefrontal cortex of OBX animals was found: chronic fluoxetine fully reversed this increase. Interestingly, previous studies have demonstrated an elevated CB_1 receptor –mediated [^{35}S]GTPγS binding in cortical samples from depressed patients[16]. Our results, in addition to validate the OBX as a model of depression, strongly support the involvement of EC signaling in both depression and antidepressant mechanisms, as it is illustrated by the absence of modifications in the animals receiving chronic fluoxetine. In this regard, it has been shown that CB_1 receptor knock-out mice exhibit enhanced depressive-like behaviours[17] and, consistently, acute low doses of cannabinoids produce antidepressant-like effects in rodents[18-19] likely via promoting hippocampal neurogenesis[20]. Nevertheless, further studies are required in order to fully clarify the role of EC system in depression.

Modulation of neural plasticity circuitry: supporting a trophic response for antidepressants

In the last few years, the interest about the mechanisms of action of antidepressants has moved from the receptor level to the intracellular signaling cascades[21]. In this regard, the cAMP-CREB transduction pathway has been consistently

implicated in the long-term effects of antidepressants. Studies carried out in postmortem samples from depressed patients have resulted in contradictory results[22]: we have also addressed this issue, finding no significant change in the basal activity of the enzyme (adenylate cyclase) in brain samples from a well characterized group of depressed patients, with respect to matched controls[9]. However, we found a significant lower response to β_1-adrenoceptors agonist-stimulated AC activity in the major depression group ($p < .01$) (see Figure 2B).

It is now well documented that chronic AD treatment enhances cell proliferation in adult rodent subgranular zone (SGZ) of hippocampus and that the time required for the differentiation and maturation of newborn neurons correlates well with the appearance of clinical response to the AD treatment[23]. In line with the new trophic hypothesis, we have addressed in detail the modifications induced by the chronic administration of the SNRI venlafaxine (40 mg/kg, 14 days) on two intracellular proteins involved in neural plasticity: in addition to the expression of CREB and pCREB, widely suggested to be involved in AD-induced cellular changes, we have also analyzed the expression of β-catenin, an emerging candidate to play a key role in neuroproliferative processes. Although the involvement of CREB and pCREB expression in the cellular responses induced by antidepressants is widely accepted, the nature of this modulation appears to depend on several factors (type of antidepressant, doses, route of administration) and contradictory data have been published[24-25]. We have found no modification in CREBN and pCREB expression in the hippocampus of rats treated with venlafaxine (figure 2D). In contrast, preliminary data from our lab appear to indicate that chronic fluoxetine does up-regulate pCREB in the rat brain.

Wnt-β-catenin cascade regulates the hippocampal neurogenesis in the adult brain. Activation of the canonical Wnt pathway leads to the inhibition of GSK-3,

allowing β-catenin to be translocated to the nucleus, where activates transcription of target genes. We have demonstrated that chronic venlafaxine induces a significant increase in the expression of β-catenin in the SZH (figure 2E): an increase in cell proliferation, quantified by BrdU immucytochemistry, is observed in the same animals[10]. Western blot (figure 2C) and immunoelectron microscopy studies have demonstrated an increased presence of β-catenin (+88.0±9 %, western data) at the nuclear level[10]. These results suggest that the hippocampal proliferative effect of chronic venlafaxine, only evident at a dose that inhibits both 5-HT and NE reuptake systems, requires a strong activation of intracellular signaling through Wnt[26], probably resulting in an increase of the expression of cell cycle regulator genes.

Conclusion

In conclusion, our group is focusing its efforts in the analysis of the modulatory changes occurring on the monoaminergic neurotransmission following antidepressant treatments, and on the modification of those involving neuroplastic and proliferative pathways. We are also interested in the possible interactions between these two types of responses. These studies may contribute to the development of new therapeutic targets for the depressive disorders, which is the ultimate goal of all our work.

Acknowledgment

We would like to thank the technical assistance of Ms. Josefa Castillo, Ms. Paula Diez del Valle, Ms. Olga Gutierrez, Ms. Lourdes Lanza, Ms. Rebeca Madureira, Ms. Alicia Martín, Ms. Beatriz Romero and Ms. Isabel Ruiz. We would like to warmly thank the contributions of Drs. Carmen del Arco, Elena del Olmo and Ricardo Mostany, former members of the group. The scientific collaboration of Prof. J. Meana (University

of the Basque Country) is specially recognized. The results reviewed in this contribution have been supported by the Spanish Ministry of Science (SAF04-00941 and SAF07-61862).

References:

1. Wong ML, Licinio J. Research and treatment approaches to depression. *Nat Rev Neurosci* 2001; 2:343-351.
2. Stahl SM, Grady MM. Differences in mechanism of action between current and future antidepressants. *J Clin Psychiatry* 2003; 13:13-17
3. Adell A, Castro E, Celada P, Bortolozzi A, Pazos A, Artigas F. Strategies for producing faster acting antidepressants. *Drug Discov Today* 2005; 10:578-585.
4. Blier P, de Montigny C. Current advances and trends in the treatment of depression. *Trends Pharmacol Sci* 1994; 15:220-226.
5. Artigas F, Romero L, de Montigny C, Blier P. Acceleration of the effect of selected antidepressant drugs in major depresión by 5-HT1A antagonists. *Trends Neurosci* 1996; 19:178-383.
6. Nestler EJ, Barrot M, DiLeone RJ, Eisch AJ, Gold SJ, Monteggia LM. Neurobiology of depression. *Neuron* 2002; 34:13-25.
7. Berton O, Nestler EJ. New approaches to antidepressant drug discovery: beyond monoamines. *Nat Rev Neurosci* 2006; 7:137-151.
8. Castro ME, Diaz A, del Olmo E y Pazos A. Chronic fluoxetine induces opposite changes in G protein coupling at pre and postsynaptic 5-HT1A receptors in rat brain. *Neuropharmacology* 2003; 44:93-101.
9. Valdizán EM, Gutierrez O, Pazos A. Adenylate cyclase activity in postmortem brain of suicide subjects: reduced response to beta-adrenergic stimulation. *Biol Psychiatry* 2003; 54:1457-1464.

10. Mostany R, Valdizán EM, Pazos A. A role for nuclear beta-catenin in SNRI antidepressant-induced hippocampal cell proliferation. *Neuropharmacology* 2008; 55: 18-26.
11. Rodríguez-Gaztelumendi A, Rojo ML, Pazos A, Díaz A. Altered CB(1) receptor-signaling in prefrontal cortex from an animal model of depression is reversed by chronic fluoxetine. *J Neurochem* 2009; (*in press*)
12. Hervás I, Vilaró MT, Romero L, Scorza MC, Mengod G, Artigas F. Desensitization of 5-HT(1A) autoreceptors by a low chronic fluoxetine dose effect of the concurrent administration of WAY-100635. *Neuropsychopharmacology* 2001; 24:11-20.
13. Hensler JG. Differential regulation of 5-HT1A receptor-G protein interactions in brain following chronic antidepressant administration. *Neuropsychopharmacology* 2002; 26:565-573.
14. Béïque JC, de Montigny C, Blier P, Debonnel G. Effects of sustained administration of the serotonin norepinephrine reuptake inhibitor venlafaxine: II. In vitro studies in the rat. *Neuropharmacology* 2000; 39:1813-1822.
15. Kelly JP, Wrynn AS, Leonard BE. The olfactory bulbectomized rat as a model of depression: an update. *Pharmacol Ther* 1997; 74:299-316.
16. Hunglung BL, Vinod KY, Kassir SA, Basavarajappa BS, Yalamanchili R, Cooper TB, Mann JJ, Arango V. Upregulation of CB1 receptors and agonist-stimulated [^{35}S]GTPγS binding in the prefrontal cortex of depressed suicide victims. *Mol Psychiatry* 2004; 9:184-190.
17. Martin M, Ledent C, Parmentier M, Maldonado R, Valverde O. Involvement of CB1 cannabinoid receptors in emotional behaviour. *Psychopharmacology (Berl)* 2002; 159:379-387.

18. Hill MN, Gorzalka BB. Pharmacological enhancement of cannabinoid CB(1) receptor activity elicits an antidepressant-like response in the rat forced swim test. *Eur Neuropsychopharmacol* 2005; 15:593-599.
19. Bambico FR, Katz N, Debonnel G, Gobbi G. Cannabinoids elicit antidepressant-like behavior and activate serotonergic neurons through the medial prefrontal cortex. *J Neurosci* 2007; 27:11700-11711.
20. Jiang W, Zhang Y, Xiao L, Van Cleemput J, Ji SP, Bai G, Zhang X. Cannabinoids promote embrionic and adult hippocampus neurogenesis and produce anxiolytic- and antidepressant-like effects. *J Clin Invest* 2005; 115:3104-3116.
21. Racagni G, Popoli M. Cellular and molecular mechanisms in the long-term action of antidepressants. *Dialogues Clin Neurosci* 2008; 10:385-400.
22. Blendy JA. The role of CREB in depression and antidepressant treatment. *Biol Psychiatry* 2006; 59:1144-1150.
23. D'Sa C, Duman RS. Antidepressants and neuroplasticity. *Bipolar Disord* 2002; 4:183-194.
24. Manier DH, Shelton RC, Sulser F. Noradrenergic antidepressants: does chronic treatment increase or decrease nuclear CREB-P?. *J Neural Transm* 2002; 109:91–99.
25. Tiraboschi E, Tardito D, Kasahara J, Moraschi S, Pruneri P, Gennarelli M, Racagni G, Popoli M. Selective phosphorylation of nuclear CREB by fluoxetine is linked to activation of CaM kinase IV and MAP kinase cascades. *Neuropsychopharmacology* 2004; 29:1831–1840.

26. Madsen TM, Newton SS, Eaton ME, Russell DS, Duman RS. Chronic electroconvulsive seizure up-regulates β-catenin expression in rat hippocampus: role in adult neurogenesis. *Biol Psychiatry* 2003; 54:1006-1014.

Figure 1.

Effect of chronic fluoxetine treatment (10mg/kg/day, 21 days, p.o) on **(A)** 5-HT_{1A} receptor-mediated stimulation of [^{35}S]GTPγS binding induced by 8-OH-DPAT (10 μM). A significant desensitization is observed at dorsal raphe (DRN) autoreceptors, but not at postsynaptic receptors (CA1) **(B)** the 5-HT_{1B} receptor-mediated stimulation of [^{35}S]GTPγS binding induced by 5-HT (10 μM) in coronal sections of the rat brain. A pattern of decrease is observed following fluoxetine. CA1: CA1 field of hippocampus; DG: dentate gyrus; Ent: entorhinal cortex; DRN: dorsal raphe nucleus; CP: caudate putamen VP: ventral pallidum; LGP: lateral globus pallidus; SN: substantia nigra. (*p< 0.05, Student t-test unpaired data).

Figure 2.

A. Effect of OBX (olfactory bulbectomiced rat) and chronic treatment with fluoxetine (10 mg/kg/day, 14 days, minipumps) or vehicle on CB_1 receptor-mediated stimulation of [^{35}S]GTPγS binding induced by WIN 55,212-2 (10μM) in rat prefrontal cortical membranes. Note that chronic fluoxetine reversed the increased functionality of CB_1 receptors induced by OBX (inserted graph). Data represent the mean ± SEM. Modified from Rodriguez-Gaztelumendi *et al*., 2009.

B. Effect of increasing concentrations of the specific β-adrenoceptor agonist xamoterol (1, 10, and 100 μM) on cAMP (cyclic adenosine monophosphate) levels (expressed as mean ± SEM of the percentage of increase over the basal) in crude membranes from postmortem human frontal cortex of control (open bars) and major depression disorder (closed bars). **p<0.01 *post hoc* paired t test after repeated-measures analysis of variance. Taken from Valdizán *et al*., 2003.

C. Effect of chronic venlafaxine (40 mg/kg/day, 14 days, minipumps) treatment on the expression of main effector proteins of Wnt and AKT/PKB signaling pathways. Graphs represent relative densitometry levels of β-catenin in treated animals as a percentage of these proteins in saline group animals (mean ± SEM). Venlafaxine treatment increases β-catenin immunoreactivity in TCL (total cell lysate), and NF (nuclear fraction) of rat

hippocampus in Western blot studies. Densitometric measurement levels were normalized to actin protein amounts.

D. Effect of chronic venlafaxine (40 mg/kg/day, 14 days, minipumps) treatment on the expression of CREB and pCREB. Graphs represent relative densitometry levels of CREB and pCREB, and pCREB/CREB ratio related to the vehicle group (mean ± SEM) of nuclear fractions from rat hippocampus from saline and venlafaxine treated animals in Western blot studies. Densitometric measurement levels were normalized to actin protein amounts.

E. β-catenin immunohistochemistry in the subgranular zone (SGZ) of the hippocampus of adult rat from vehicle and venlafaxine (40 mg/kg/day, 14 days, minipumps) treated animals. β-catenin positive cells were labelled using DAB as a chromogen. Cresyl violet was used as counter-staining. The number of β-catenin positive cells in the SGZ is significantly increased after chronic treatment with venlafaxine.