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DIFFERENTIAL *IN VIVO* REGULATION OF TH AND DBH MRNA IN RAT ATRIA BY MAPROTILINE AND FLUOXETINE

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Abstract - It is well known that antidepressants affect central monoaminergic neurotransmission and that they also modulate hormone release in peripheral tissues. Repeated maprotiline (a noradrenaline reuptake inhibitor) and fluoxetine (a serotonin reuptake inhibitor) treatment on gene expression of the catecholamine biosynthetic enzymes were examined in rat atria and ventricles *in vivo*. Maprotiline decreased the gene expression of tyrosine hydroxylase (TH) and dopamine-ß-hydroxylase (DBH) in the rat atrium. Fluoxetine increased gene expression of TH and DBH, but not of phenylethanolamine N-methyltransferase (PNMT). Chronic application of antidepressants did not change the expression of these enzymes in the ventricles. We conclude that repeated administration of fluoxetine enhances gene transcription of TH and DBH and subsequently stimulates noradrenaline synthesis in rat atria *in vivo*.

Key words: Antidepressants, catecholamine enzymes, gene expression, atria, ventricles, rats

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INTRODUCTION

Serotonin and noradrenaline are involved in the mechanism of action of most antidepressants. Noradrenergic and serotonergic antidepressants have been associated with somewhat different clinical effects. Maprotiline, a noradrenaline reuptake inhibitor and fluoxetine, a serotonin reuptake inhibitor, are widely used antidepressants. Recently we demonstrated that both antidepressants resulted in a significant reduction of the symptoms of depression. Long-term maprotiline treatment produced significant increases in plasma noradrenaline, while long-term fluoxetine treatment was followed by higher levels of noradrenaline and adrenaline (Dronjak et al., 2007; Spasojević et al., 2008). A number of studies have investigated the effect of antidepressants on catecholamine biosynthesis. Studies have shown that most of the classical antidepressants do not stimulate catecholamine synthesis. As to the acute effects of tricyclic antidepressants on tyrosine hydroxylase (TH) activity, chronic treatment with classical antidepressants has been found to have little effect or an inhibitory effect on the activity and expression of TH in the rat brain *in vivo* (Cambell et al., 1979; Nestler et al., 1990; Moret and Briley, 1992). However, Zhu et al. (2005) showed that repeated treatment of rats with desipramine increased TH mRNA levels in the locus coeruleus and amygdala. In contrast, repeated administration of fluoxetine decreased TH gene expression in the ventral tegmental area and substantia nigra (Oliva et al., 2005). These findings indicate that not all types of antidepressants produce consistent changes in TH mRNA levels

in the brain in response to chronic treatment. While these findings suggest that chronic antidepressant treatments can alter the synthesis of catecholamines in the brain, no consistent reproducible effect of these treatments on the biosynthetic pathway of catecholamine in peripheral tissues have been presented.

The rate-limiting enzyme in the synthesis of catecholamine is TH which catalyzes the conversion of tyrosine to dihydroxyphenylalanine (DOPA). DOPA is converted to dopamine by a nonspecific enzyme, aromatic L-amino acid decarboxylase (AAAD). Dopamine is taken up from the cytoplasm into storage vesicles and converted into noradrenaline by dopamine-ß-hydroxylase (DBH). Noradrenaline is then converted to adrenaline by the soluble cytoplasmic enzyme phenylethanolamine N-methyltransferase (PNMT) (Kvetnansky et al., 2009). In a previous study, we found that repeated administration of fluoxetine enhanced gene transcription of TH and DBH in adrenal medulla, whereas maprotiline did not change gene expression of these enzymes (Spasojevic et al., 2010). This prompted us to investigate the changes in gene transcription of catecholamine biosynthetic enzymes in right and left atria and ventricles after repeated antidepressants treatment. To estimate the influence of long-term treatments with noradrenergic and serotonergic reuptake inhibitors on the genes that encode the catecholamine biosynthetic enzymes, we compared the effects of maprotiline and fluoxetine on the expression of three catecholamine biosynthetic enzyme genes TH, DBH and PNMT using the TaqMan RT-PCR assay.

MATERIALS AND METHODS

Animals

Adult Wistar rat males weighing 280-320 g were used. The rats were maintained in a temperature-controlled room (21±1.0 °C) at a 12 h/12 h light/dark cycle. Care was taken to minimize the pain and discomfort of the animals that were treated according to the recommendations of the Ethical Committee of the "Vinča" Institute, Belgrade, which are in accordance with the Guide for Care and Use of Laboratory Animals of the National Institute of Health, Bethesda, MD, U.S.A.

Drugs and chronic treatment protocols

The rats were randomly divided into three groups. The animals received daily injections of the vehicle (sterile water), maprotiline (10 mg/kg) or fluoxetine (10 mg/kg) by the i.p. route. Maprotiline (Sigma-Aldrich Chemie, Germany) and fluoxetine (Aeigis LTD, Cyprus) solutions in sterile water were sonicated 10 min and were prepared *ex tempore*. The rats were killed and the right and atrial appendage and ventricles were rapidly dissected, frozen in liquid nitrogen and stored at -70^{0}C until use.

Real-time RT-PCR

Total RNAs were isolated using TRIZOL reagent (Invitrogen, CA, U.S.A.). Reverse transcription was performed employing the Ready-To-Go You-Prime First-Strand Bead (AP, Biotech) and pd $(N)_6$ primer according to manufacturer's protocol. Real-Time RT-PCR assay was performed exactly as previously described (Gavrilović et al., 2008). PCR was performed in the ABI Prism 7000 Sequence Detection System at 50^{o}C for 2 min, 95^{o}C for 10 min, followed by 40 cycles at 95^{o}C for 15 sec and 60^{o}C for 1 min. A reference, endogenous control was included in each analysis to correct for the differences in inter-assay amplification efficiency, and all transcripts were normalized to cyclophyline A (ID:Rn 00690933) expression.

Statistical analysis

The results are presented as means ± S.E.M. The statistical significance of the differences in gene expression levels of the examined catecholamine biosynthetic enzymes was estimated by the One-way ANOVA test. The Tukey *post-hoc* test was used to evaluate the differences between the groups. Statistical significance was accepted at $p<0.05$.

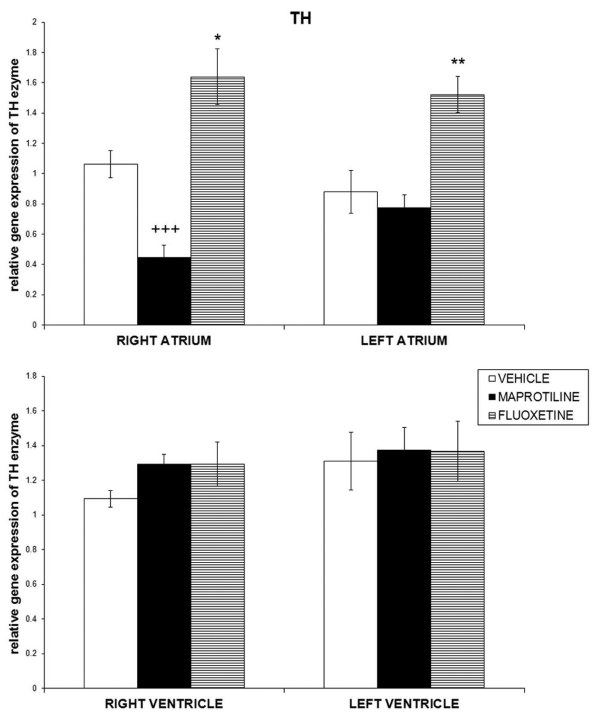

Fig. 1. The effects of chronic treatments with maprotiline and fluoxetine on tyrosine hydroxylase (TH) mRNA levels in the right and left heart atria and ventricle of adult rat males. The values are means ± S.E.M. (6-8 rats). Statistical significance: +++ p<0.001 placebo *vs.* maprotiline, * p<0.05, ** p<0.01 placebo *vs.* fluoxetine.

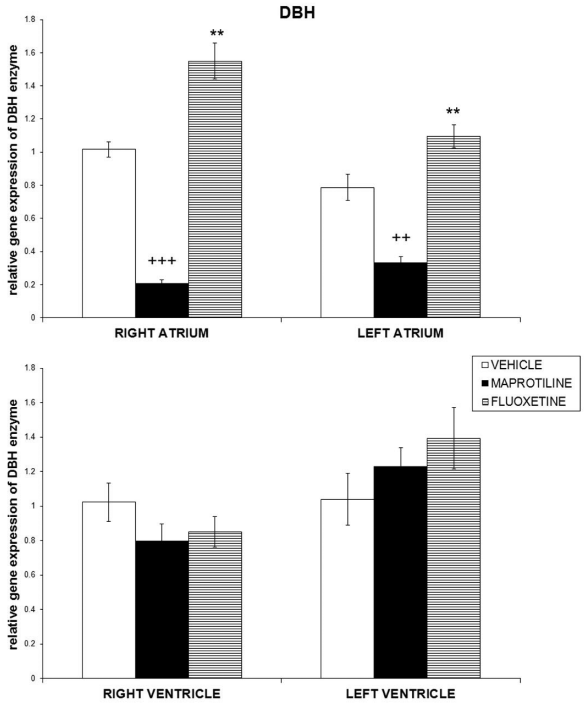

Fig. 2. The effects of chronic treatments with maprotiline and fluoxetine on dopamine-ß-hydroxylase (DBH) mRNA in the right and left heart atria and ventricle of adult rat males. The values are means ±S.E.M. (6-8 rats). Statistical significance: ++ p<0.01, +++ p<0.001 placebo *vs.* maprotiline, ** p<0.01 placebo *vs.* fluoxetine.

RESULTS

Antidepressant treatments substantially influenced the expression of TH only in the atria. Maprotiline reduced the expression of this enzyme by 58% (p<0.001) in the right atrium. The antidepressant treatment had no effect on the level of TH mRNA in the left atrium. Fluoxetine, unlike maprotiline, significantly increased the relative rate of gene transcription of this enzyme in the right atrium by 54% (p<0.05) and in the left atrium by 73% (p<0.01). Chronic application of antidepressants did not change the expression of TH in the ventricles (Fig 1).

One-way ANOVA analysis revealed significant antidepressant-induced variations of DBH mRNA levels in the atria, but no significant effects in the ventricles. Treatment with maprotiline decreased (in the right atria by 80%, p<0.0001, and in the left atria by 58%, p<0.01), while fluoxetine increased DBH mRNA levels (in the right atria by 58%, p<0.001, and in the left atria by 39%, p<0.01), similarly to the effect that they had on the expression of TH (Fig. 2). However, chronic application of either maprotiline or fluoxetine did not affect PNMT mRNA levels in cardiac tissue (Fig. 3).

DISCUSSION

This is the first study to examine the effect of repeated antidepressant treatments on the expression of genes encoding for three catecholamine biosynthetic enzymes in the right and left atrial appendages and ventricles of the rat *in vivo*. We found that repeated treatment of fluoxetine led to an increase in TH and DBH mRNA levels, whereas

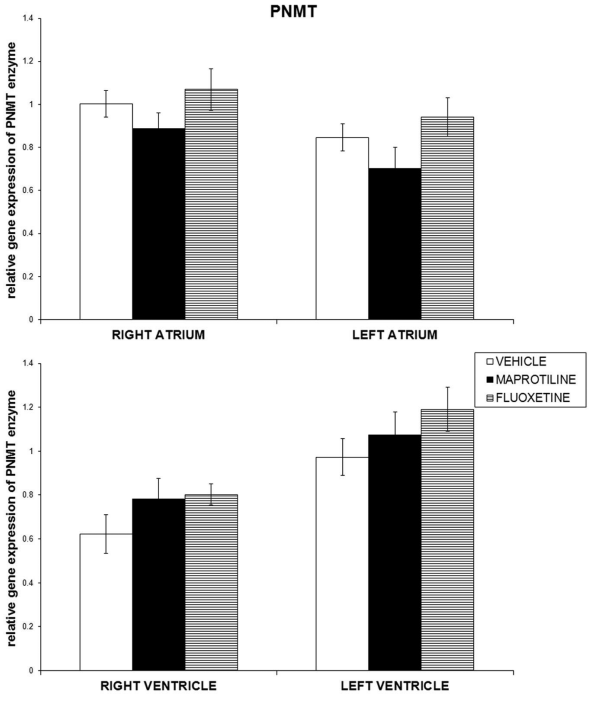

Fig. 3. The effects of chronic treatments with maprotiline and fluoxetine on phenylethanolamine N-methyltransferase (PNMT) mRNA levels in the right and left heart atria and ventricle of adult rat males. The values are means ±S.E.M. (6-8 rats).

maprotiline treatment decreased the transcription of these enzymes in atria. However, PNMT mRNA in the atria remained unchanged after both repeated maprotiline and fluoxetine treatments. On the other hand, in the right and left ventricles, neither maprotiline nor fluoxetine had any effect on the transcription of genes encoding for either enzyme. This result is the first direct evidence for a stimulatory effect of fluoxetine and a suppressive effect of maprotiline on catecholamine synthesis in the atria. The different effects on the regulation of TH and DBH in atria during maprotiline and fluoxetine treatment could be due to the different effects of the cAMP response element-binding (CREB) on gene transcription. Namely, it was confirmed that desipramine reduces the phosphorylation of CREB on Ser-119, thus inhibiting CRE-dependent gene transcription (Schwaninger et al, 1995; Manier et al, 2002) including the genes for TH and DBH. In contrast, chronic application of SSRI drugs increase the expression of CREB in rat brain (Nibuya et al, 1996; Duman, 2004). Interestingly, repeated maprotiline and fluoxetine treatments did not affect TH and DBH gene transcription in the right and left ventricles. Different modes of regulation of the expression of distinct genes have been reported to exist not only in individual organs but also within a single organ, e.g. in the heart. Deindl et al. (2003) recently reported that lactate dehydrogenase-B is upregulated in the right ventricles, but downregulated in the left ventricles and atria. Also, c-Jun-N-terminal protein kinases (JNKs) are elevated in the right ventricles of rats with chronic intermittent altitude hypoxia, while their expression is downregulated in the left ventricles (Strniskova et al., 2006). Also, Zhao et al (2002) found that many genes are differentially expressed in the atria and ventricle. Shinkai et al. (2007) reported that the serotonin/noradrenaline reuptake inhibitor milinacipran activates TH through a p44/42 mitogen-activated protein kinase (MAPK)-dependent pathway in cultured bovine adrenal medullary cells. It is possible that the signaling pathways are different in tissues, which could provide a basis for selective pharmacologic interventions. Therefore, further experiments are required to examine the effects of antidepressants on the mechanisms that regulate catecholamine biosynthetic enzymes. On the other hand, we found that repeated maprotiline and fluoxetine treatments did not change the level of PNMT mRNA in all cardiac tissues. Evidence supports the importance of the activation of the hypothalamic-pituitary-adrenocortical axis in the regulation of PNMT gene expression. Glucocorticoids regulate PNMT at the transcriptional and posttranscriptional levels (Wong et al., 1992; Tai et al., 2002). We reported previously that long-term administration of fluoxetine did not increase plasma corticosterone, and this might explain unchanging PNMT mRNA levels.

Further studies are needed to confirm our results and to investigate the effect of other antidepressants on the synthesis of catecholamines in the peripheral sympathoneural system.

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