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Repeated co-treatment with fluoxetine and amantadine induces brain-derived neurotrophic factor gene expression in rats

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Abstract:
In the present study, we investigated the influence of repeated treatment with fluoxetine (FLU, 5 or 10 mg/kg) and amantadine (AMA, 10 mg/kg), given separately or jointly (twice daily for 14 day), at the mRNA level (the Northern blot) in the hippocampus and cerebral cortex. The experiment was carried out on male Wistar rats. The tissue for biochemical assays was dissected 24 h after the last dose of drug. We also studied the effect of repeated treatment with FLU and AMA on the action of 5-hydroksytryptamine ($5\text{-HT})_{1A}$- and 5-HT_2 receptor agonists (±)-8-hydroxy-2-(di-*n*-propylamino)-tetralin (8-OH-DPAT) and (±)-1-(4-iodo-2,5-dimethoxy-phenyl)-2-aminopropane ((±)DOI), respectively, in behavioral tests. The obtained results showed that FLU (10 mg/kg) in the hippocampus, and FLU (5 and 10 mg/kg) and AMA (10 mg/kg) in the cerebral cortex, significantly elevated BDNF mRNA levels. Joint administration of FLU (5 or 10 mg/kg) and AMA (10 mg/kg) induced a more substantial increase in BDNF gene expression in the cerebral cortex (but not in the hippocampus), and inhibited the behavioral syndrome induced by 8-OH-DPAT or (±)DOI (compared to treatment with either drug alone). The obtained results suggest that the enhancement of BDNF gene expression may be essential for the therapeutic effect of the co-administration of FLU and AMA in drug-resistant depressed patients, and that among other mechanisms, 5-HT_{1A} and 5-HT_2 receptors may play some role in this effect.

Key words:
repeated treatment, fluoxetine, amantadine, mRNA BDNF, 5-HT_{1A} and 5-HT_2 syndrome, rats

Introduction

It is estimated that 30–40% of patients suffering from depression do not respond to a conventional therapy. The problem of antidepressant-resistant depression has been the subject of extensive study, yet with no apparent therapeutic success. Therefore, to improve the therapy, a combination of antidepressant drugs (ADs) belonging to various pharmacological groups, or a combination of an AD and a substance enhancing its effect, is presently used in the clinic. In recent years, much attention has been devoted to the glutamatergic system in general, and to *N*-methyl-D-aspartate (NMDA) receptor antagonists in particular. The antidepressive properties of those compounds were described in the past decade [66], and antidepressant-like actions were subsequently demonstrated for competitive NMDA receptor antagonists such as CGP-37849 and AP-1, and for uncompetitive ones such as amantadine (AMA), memantine and neramexane in animal models [25, 32, 37, 39, 47, 57]. In addition, our earlier studies demonstrated that joint administration of an AD (imipramine, venlafaxine or fluoxetine – [FLU]) and an uncompetitive antagonist of NMDA receptors

(AMA) produced a more potent "antidepressant" effect in the forced swimming test (measured as the shortening of immobility time) than did treatment with either of those drugs alone [44, 47]. Positive effects were also observed when the above-mentioned drugs were used jointly in inactive doses. Moreover, a small clinical trial recently revealed that *iv* infusion of ketamine produced a short-lasting psychotomimetic effect (hours) and a long-lasting antidepressive action (days) [4, 72]. Some earlier preliminary clinical studies showed that combined treatment of drug-resistant unipolarly depressed patients with imipramine (IMI) and AMA brought about a significant clinical improvement [41, 43]. A similar result was reported by Stryjer et al. [60], who also observed in a small open-label trial that AMA given with conventional ADs effected a 50% improvement in treatment-resistant patients and, more interestingly, a decrease in the time necessary for a therapeutic improvement in some of those patients. The latter finding suggests that the results obtained with animal models may also be valid in the clinic.

Despite a search of more than 40 years, the mechanism of antidepressant action has not yet been fully elucidated. The majority of adaptive changes thought to be "responsible" for neurochemical antidepressant mechanisms are not common to all antidepressants therapies [8, 30, 56, 70, 71]. Therefore, a search for common alterations is still in progress. Numerous findings indicate a role of brain-derived neurotrophic factor (BDNF) – a member of the structurally and functionally homologous neurotrophin family – in the pathophysiology of depression, as well as in the mechanism of action of ADs. Studies conducted on postmortem human brain tissue reported an increase in BDNF immunoreactivity in patients treated with ADs compared to untreated subjects [5]. Moreover, some earlier clinical reports indicated low BDNF serum levels in patients with major depressive disorders compared to control subjects; they also indicated that antidepressant treatment increased serum BDNF levels in depressed patients up to the level described in healthy controls [2, 3, 13, 14, 50, 53]. Moreover, a number of data have shown that repeated (but not acute) administration of ADs enhanced BDNF gene expression in the hippocampus [7, 9, 10, 17, 33, 48, 49], while electroconvulsive shock and tranylcypromine, IMI or mirtazapine induced a similar effect in the hippocampus and cerebral cortex [33, 42, 46].

Since the most potent effect of ADs on BDNF gene expression was found after prolonged treatment, in the present study, we investigated the influence of repeated treatment (twice daily for 14 days) of FLU (5 or 10 mg/kg) and AMA (10 mg/kg), given separately or jointly, on BDNF mRNA levels in rat hippocampus and cerebral cortex (where the effect of joint treatment with AMA and FLU on BDNF expression had not been previously studied). The measurement was carried out 24 h after the last oral administration. The chosen time point was considered important, since an earlier study had shown that long-term systemic injection of some ADs resulted in a bi-phasic change in BDNF mRNA levels and, as a consequence, in a decrease at 4 h and an increase at 24 h after the last injection [7]. In addition, the effect of the two above-mentioned drugs on the behavioral syndrome evoked by (±)-8-hydroxy-2-(di-*n*-propylamino)-tetralin (8-OH-DPAT), a 5-hydroksytryptamine (5-HT)$_{1A}$ receptor agonist, or on the head twitches induced by (±)-1-(4-iodo-2,5-dimethoxyphenyl)-2-aminopropane ((±)DOI), a 5-HT$_2$ receptor agonist, was measured 24 h after the last dose of FLU or AMA (or after their single administration).

Materials and Methods

Animals and drug administration

The experiments were carried out on rats (male Wistar, 250–270 g). The animals had free access to food and water before the experiment, and were kept on a 12-h light/dark cycle (the light on at 07:00) at a constant room temperature (22 ± 1°C). FLU at doses of 5 or 10 mg/kg, and AMA at a dose of 10 mg/kg, were dissolved in distilled water and administered repeatedly (twice daily *po* for 14 days), separately or jointly (at a volume of 2 ml/kg). Twenty-four hours after the last treatment, the rats were decapitated, their hippocampi and cerebral cortices were dissected, immediately frozen on dry ice and stored until they were used for biochemical assays. The behavioral syndrome evoked by 8-OH-DPAT, a 5-HT$_{1A}$ receptor agonist, or the head twitches induced by (±)DOI, a 5-HT$_2$ receptor agonist, were measured 24 h after the last dose of IMI and AMA (or after their single administration). The animals were used only once in each experiment.

All experimental protocols were approved by the Local Bioethics Commission for Animal Experiments

at the Institute of Pharmacology, Polish Academy of Sciences in Kraków.

Determination of BDNF mRNA

The procedure for determining BDNF mRNA levels was carried out according to Legutko et al. [20]. Briefly, total RNA was extracted by a chaotropic lysis (TRIzol Reagent, Life Technologies) following the manufacturer's protocol. Northern blot analysis was carried out using ~7 μg of total RNA, which was separated on a 1% denaturing agarose-formaldehyde gel, and subsequently transferred on to a nylon membrane (Nytran, Schleicher and Schuell) and immobilized by ultraviolet (UV) radiation. A probe for rat BDNF was generated from cDNA by polymerase chain reaction (PCR), using the following primers: 5′-ACT-CTG-GAG-AGC-GTG-AAT-GG-3′ and 5′-CAG-CCT-TCC-TTC-GTG-TAA-CC-3′; the 470 bp product was cloned into the pCRII TA cloning vector. The insert cut with the enzyme EcoRI was randomized by a primer-labeled with P^{32}dCTP and purified (Prime-It Rmt, Stratagene). Hybridization was performed overnight in Church's buffer at 65°C. The hybridized filters were washed for 30 min in 2 × saline-sodium citrate (SSC) buffer/0.1% sodium dodecyl sulfate (SDS) at room temperature, and for 30 min in 0.1 × SSC/0.1% SDS at 55°C and then exposed. The same filters, stripped of the BDNF probe (washed three times in 0.1 × SSC/0.1% SDS at 100°C for 10 min), were re-hybridized for the β-action cDNA probe (Clontech) to normalize RNA loading. The Northern blots were quantified using digitalized autoradiographs (PhosphorImager, Image 4.0 Fuji). Each group consisted of six rats.

Behavioral syndrome induced by 8-OH-DPAT

Separate groups of rats were used for Northern blot analyses and behavioral measurements. 8-OH-DPAT (5 mg/kg, *ip*) was given 24 h after the last dose of FLU or AMA (or after their single administration). Immediately after the injection of 8-OH-DPAT, the animals were placed separately in cages. Observation sessions began 3 min after 8-OH-DPAT injection and were repeated every 3 min for a period of 15 min. Reciprocal forepaw treading and flat body posture were scored using a ranked intensity scale (0 point – absence; 1 point – equivocal; 2 points – present; 3 points – intense). Each score was summed over five observation periods [65]. Each group consisted of six rats.

Head twitches induced by (±)DOI

(±)DOI (2.5 mg/kg, *ip*) was given 24 h after the last dose of FLU or AMA (or after their single administration). Head twitches were recorded immediately after (±)DOI administration, and the recording was continued for 30 min. Each group consisted of 6 rats.

Drugs

Amantadine hydrochloride (Sigma, USA; AMA), fluoxetine hydrochloride (Farmacom, Kraków, Poland; FLU), (±)-8-hydroxy-2-(di-*n*-propylamino)-tetralin hydrobromide (Research Biochemicals Int., USA; 8-OH-DPAT) and (±)-1-(4-iodo-2,5-dimethoxyphenyl)-2-aminopropane hydrochloride (Research Biochemicals Int., USA; (±)DOI) were used in the present study.

Statistical analysis

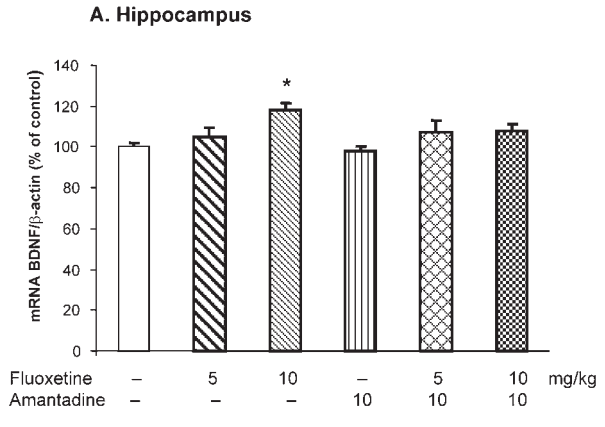
The obtained data were evaluated using a one-way analysis of variance (ANOVA) followed, when appropriate, by individual comparisons with the control using Dunnett's test.

Results

Determination of BDNF mRNA

The effect of repeated treatment with FLU (5 or 10 mg/kg) and AMA (10 mg/kg), administered separately or jointly, on BDNF mRNA levels in rat hippocampus and cerebral cortex is shown in Figure 1. In the hippocampus, repeated treatment with FLU at the higher dose (10 mg/kg) significantly elevated BDNF mRNA levels (by 18.1%) compared to the vehicle-treated control. Neither FLU administered repeatedly at the lower dose (5 mg/kg), nor AMA (10 mg/kg) significantly changed BDNF mRNA expression. Co-administration of FLU (5 or 10 mg/kg) and AMA (10 mg/kg) did not affect the level of BDNF mRNA in a statistically significant manner compared to treatment with either drug alone [ANOVA; $F(5, 30) = 5.30$, $p < 0.001$; Fig. 1A].

In rat cerebral cortex, repeated administration of FLU (5 or 10 mg/kg) and AMA (10 mg/kg) increased BDNF mRNA levels compared to the vehicle-treated



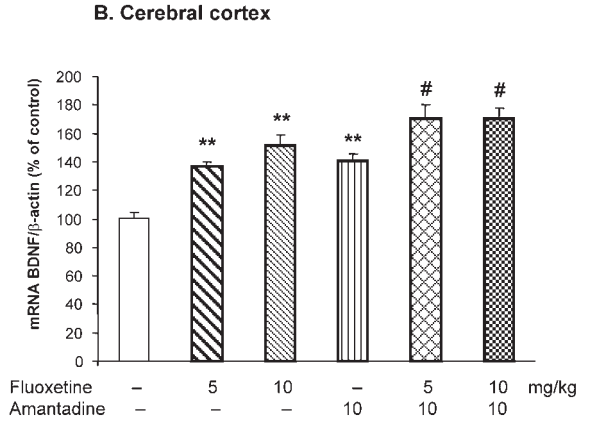
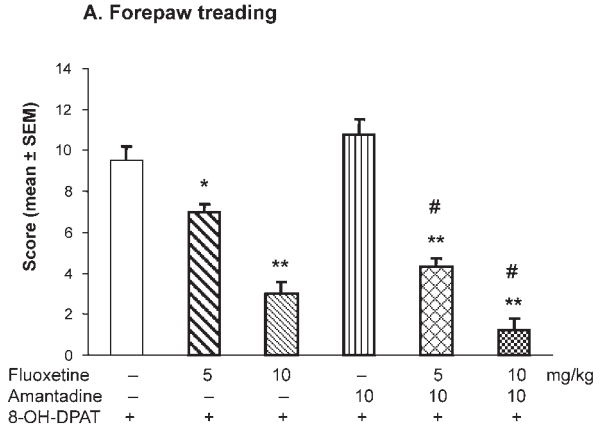


Fig. 1. The effect of repeated co-treatment with fluoxetine (FLU) and amantadine (AMA) on BDNF mRNA levels (Northern blot) in the hippocampus **(A)** and cerebral cortex **(B)** in rats, measured 24 h after the last dose of FLU or AMA. The results are presented as the mean ± SEM of 6 animals/group. The data were statistically evaluated by ANOVA, followed by individual comparisons using Dunnett's test. * $p < 0.05$, ** $p < 0.001$ *vs.* vehicle group, # $p < 0.001$ *vs.* appropriate FLU or AMA group



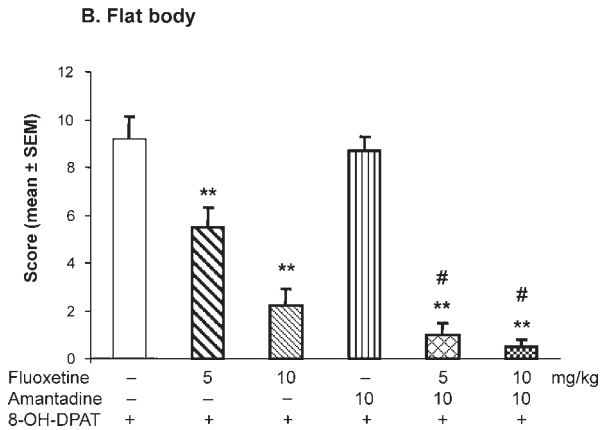


Fig. 2. The effect of repeated co-treatment with fluoxetine (FLU) and amantadine (AMA) on the 8-OH-DPAT-induced behavioral syndrome [forepaw treading **(A)** and flat body **(B)**] in rats. The test was carried out 24 h after the last dose of FLU and AMA. 8-OH-DPAT (5 mg/kg) was injected directly before the test. The results are presented as the mean ± SEM of 6 animals/group. The data were statistically evaluated by ANOVA, followed by individual comparisons using Dunnett's test. * $p < 0.05$, ** $p < 0.001$ *vs.* 8-OH-DPAT group, # $p < 0.001$ *vs.* appropriate FLU + 8-OH-DPAT or AMA + 8-OH-DPAT group

control (by 36.9, 51.6 and 40.6%, respectively). Co-administration of FLU (5 or 10 mg/kg) and AMA (10 mg/kg) induced a more potent (statistically significant) increase in the level of BDNF mRNA than did treatment with either drug alone [ANOVA; $F(5, 30) = 15.01$, $p < 0.001$; Fig. 1B].

Behavioral syndrome induced by 8-OH-DPAT

Neither FLU (5 and 10 mg/kg) or AMA (10 mg/kg), given separately or jointly in a single dose, changed the behavioral syndrome induced by the 5-HT_{1A} agonist 8-OH-DPAT (5 mg/kg) (data not shown). Repeated administration of FLU in both of the tested doses (5 and 10 mg/kg) inhibited the behavioral effect induced by 8-OH-DPAT in a statistically significant manner. Co-treatment with FLU (5 or 10 mg/kg) and AMA (10 mg/kg) induced more potent (statistically significant) inhibition of 8-OH-DPAT action [forepaw treading – ANOVA; $F(5, 30) = 49.05$, $p < 0.001$ (Fig. 2A); flat body posture – ANOVA; $F(5, 30) = 36.60$, $p < 0.001$ (Fig. 2B)].

Head twitches induced by (±)DOI

When given either separately or jointly in a single dose, neither FLU (5 and 10 mg/kg) nor AMA (10 mg/kg) affected the head twitch reaction induced by the

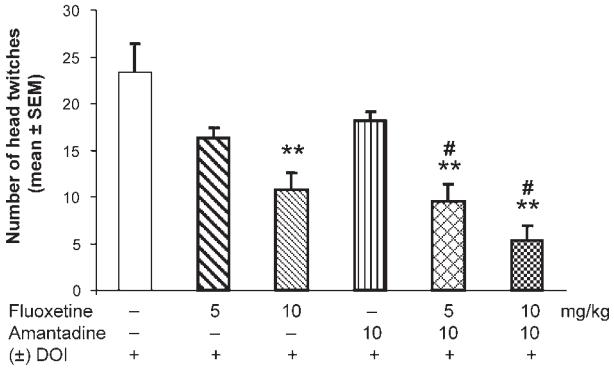


Fig. 3. The effect of repeated co-treatment with fluoxetine (FLU) and amantadine (AMA) on (±)DOI-induced head twitches in rats. The test was carried out 24 h after the last dose of FLU and AMA. (±)DOI (2.5 mg/kg) was injected directly before the test. The results are presented as the mean ± SEM of 6 animals/group. The data were statistically evaluated by ANOVA, followed by individual comparisons using Dunnett's test. ** $p < 0.001$ *vs.* (±)DOI group, # $p < 0.001$ *vs.* appropriate FLU + (±)DOI or AMA + (±)DOI group

5-HT_{2A} agonist (±)DOI (2.5 mg/kg) (data not shown). Repeated administration of FLU at the higher dose (10 mg/kg) inhibited the behavioral effect induced by (±)DOI. Co-treatment with FLU (5 or 10 mg/kg) and AMA (10 mg/kg) induced more potent (statistically significant) inhibition of the head twitches induced by (±)DOI than did treatment with IMI or AMA alone [ANOVA; $F(5, 30) = 12.09$, $p < 0.001$; Fig. 3].

Discussion

The aim of the present study was to investigate the influence of repeated treatment (twice daily for 14 days) with FLU (5 or 10 mg/kg) and AMA (10 mg/kg), given separately or jointly, on BDNF mRNA levels in rat hippocampus and cerebral cortex. The obtained results indicated that FLU given repeatedly increased BDNF gene expression in both brain regions examined (the hippocampus and cerebral cortex). The effect of FLU in that test was similar to that produced by MAOIs, tranylcypromine, electroconvulsive shock (ECS) [33] or mirtazapine [46]. Moreover, AMA significantly increased BDNF mRNA levels in the cerebral cortex only. In addition, Marvanova et al. [31] also showed that memantine, an NMDA receptor antagonist, produced an increase in BDNF mRNA expression in various brain areas, but the effect was studied after acute administration only. A similar increase in BDNF expression following acute administration was found for other NMDA receptor antagonists including MK-801 (dizocilpine), ifenprodil [21, 28, 64] and ketamine [12]. Furthermore, the present study showed that co-administration of FLU (5 or 10 mg/kg) and AMA induced a more potent increase in the level of BDNF mRNA BDNF gene expression in the cerebral cortex than did treatment with either of those drugs alone. Additionally, other research has identified BDNF and 5-HT as two prominent signals acting in concert to regulate neuronal plasticity in a number of brain regions. These two signals co-regulate each other in such a way that 5-HT stimulates the expression of BDNF, while BDNF enhances the growth and survival of 5-HT neurons. The impaired 5-HT and BDNF signalling is pivotal to depression and anxiety disorders, but may also play an important role in the pathogenesis of several age-related disorders [29]. Our present study indicates that repeated co-treatment with FLU and AMA inhibits 5-HT_{1A} and 5-HT_2 neurotransmission in behavioral tests more potently than does FLU alone, and that neither acute nor chronic administration of AMA significantly alters this effect. Moreover, we previously observed a decrease in the density of 5-HT_2 receptors after repeated treatment with FLU [24] and other ADs [22, 23] in biochemical and behavioral experiments. Recently, using *in vivo* microdialysis, it has been found that AMA or budipine (another NMDA receptor antagonist), given acutely with ADs (reboxetine, paroxetine or clomipramine), elevate extracellular 5-HT concentration in the frontal cortices of freely moving rats. Repeated co-treatment with AMA or budipine and ADs (reboxetine, paroxetine or clomipramine) facilitate and potentiate these effects. In addition, neither acute nor chronic (7, 14, and 21 days) administration of AMA or budipine significantly changes the extracellular 5-HT concentration in the frontal cortices of freely moving rats [36]. The latter authors suggested that the above-described effect on 5-HT concentrations may be caused by a number of factors, including alteration of the glutamatergic tone at NMDA receptors, which may facilitate 5-HT neurotransmission, as well as the action of budipine and AMA on monoamine metabolism. In addition, the role of 5-HT_{2A} receptor-mediated regulation of BDNF mRNA in the hippocampus and the neocortex, and in the stress-induced down-regulation of BDNF expression in the hippocampus, was measured by Vaidya et al. [67, 68]. The above results indicated that regula-

tion of BDNF expression by 5-HT_{2A} receptor agonists, but not by 5-HT_{1A} receptor agonists, lead to adaptations of synaptic strength in the hippocampus, and the neocortex may mediate some of the acute and long term behavioral effects of these agents, and that 5-HT_{2A} receptors mediate, at least in part, the stress-induced down-regulation of BDNF expression in the rat hippocampus.

On the other hand, AMA appears to act *via* several pharmacological mechanisms, none of them being identified as the chief mode of action. Moreover, AMA exhibits dopaminergic, noradrenergic and serotonergic activities, blocks monoamine oxidase A and NMDA receptors, and probably elevates β-endorphin/β-lipotropin levels [16]. It is still not clear which of these mechanisms are relevant at the therapeutic dose of the drug. All these actions may be crucial for antidepressant properties, hence, it has been suggested that AMA is likely to act as an antidepressant through not one, but several mechanisms thought to be related to antidepressant activity [16]. For example, it cannot be excluded that the antagonistic action of AMA at NMDA receptors may constitute an additional mechanism of antidepressant activity [4].

Unfortunately, in the case of NMDA receptor antagonists, many of these compounds are of very limited value to patients, partly due to extremely poor CNS penetration in some cases, or unacceptable side effects in others [4]. It seems that the clinical tolerability of uncompetitive NMDA receptor antagonists may depend on their low affinity and, possibly, subunit specificity within the NMDA complex [40]. AMA, a weak NMDA receptor antagonist that is currently used in the clinic, has been shown to have a synergistic effect when given with conventional ADs in animal models of depression [47], while the above-described combination has a beneficial effect on treatment-resistant patients [41, 43, 60].

Robust increases in the levels of BDNF mRNA in the hippocampal and cortical regions have been reported following repeated administration of ADs to rats [7, 10, 33]; however, some other studies indicate that these phenomena are not common to all ADs. For example, although tranylcypromine and ECS increase BDNF mRNA levels [7, 10], more selective ADs such as desipramine and FLU have diverse effects [7, 10, 33, 73]. In addition, Larsen et al. [19] showed that BDNF mRNA levels in the dentate gyrus were increased after treatment with venlafaxine (7, 14 and 21 days) and IMI (14 and 21 days), but not after treatment with FLU. A transient increase in synaptophysin mRNA was observed after treatment with venlafaxine and FLU, whereas IMI had no effect. In the CA1 region, a reduction of GAP-43 mRNA was observed after treatment with IMI (21 days) and FLU (7 and 14 days). The above result suggests that venlafaxine and IMI, but not FLU, induce neuroplastic effects in the hippocampus through stimulation of BDNF mRNA expression, and that the effect on BDNF is not directly translated into regulation of synaptophysin and GAP-43 mRNA [19]. Alterations in BDNF protein levels have been described in only a few studies [1, 59] in which tranylcypromine and ECS increase protein levels, but FLU and desipramine are without effects [1]. BDNF has also been found to be regulated by exposure to stress [58], and treatment with ADs has been shown to block this down-regulation [33, 58]. Furthermore, the duration of drug treatment and the interval following drug administration may have an impact on the overall levels of BDNF [7]. Hence, differences in the stressful states of animals, shown by various studies, in addition to the duration of treatment and the time when BDNF levels are analyzed following treatment, may account for the discrepancies between these studies.

Interestingly, a study by Nibuya et al. [34] has also demonstrated that repeated, but not acute, administration of ADs from several different classes, as well as ECS application increases the expression of cAMP response element binding protein (CREB) mRNA in the hippocampus; it has also been suggested that the transcription factor is a common intracellular target of ADs. The above results also support the hypothesis that 5-HT- and noradrenaline (NA) receptor-coupled intracellular cascades that cause CREB regulation are activated (but not down-regulated) by repeated AD treatment. The latter observation suggests that although the levels of some 5-HT and NA receptors (e.g., 5-HT_{2A}, 5-HT_7 and β-adrenergic ones) can be reduced by repeated AD treatment, the functional output of these receptor-coupled intracellular pathways is likely to increase. It is noteworthy that the up-regulation of CREB is a common effect of repeated treatment with ADs, which, in turn, leads to regulation of specific target genes such as BDNF and trkB, and to a long-term impact of these treatments on brain functions.

BDNF binds to trkB receptors in the brain. Upon activation by ligand-dependent autophosphorylation, this tyrosine kinase initiates a variety of intracellular

signalling cascades, thereby inducing the MEK-ERK pathway and the downstream activation of RSK2, which can phosphorylate CREB. A link between CREB and BDNF is strongly suggested by the findings that the AD-mediated up-regulation of BDNF is blocked in CREB-deficient mice [6]. Therefore, although CREB does not seem to mediate the acute behavioral or endocrine effects of ADs, endpoints that rely on chronic drug administration, such as changes in gene expression, are reduced when CREB function is impaired. In addition, chronic administration of FLU to rats increases phosphorylated CREB (P-CREB) levels in several brain regions, including the amygdala, cortex, dentate gyrus and hypothalamus [62]. Interestingly, desipramine increases the levels of P-CREB in the dentate gyrus only [62]. In another experiment, chronic treatment with FLU increased P-CREB levels in the prefrontal and frontal cortices to a greater extent than did the selective naradrenaline reuptake inhibitors desipramine and reboxetine [63]. Moreover, activation of the Ca^{2+} – CaM kinase IV and MAP kinase cascades contributed more substantially to that increase in P-CREB levels than did activation of the cAMP-PKA pathway. The selectivity of ADs at reuptake sites does not vary throughout the brain; however, depending on which postsynaptic receptors and associated signal transduction pathways are activated in various brain regions, a subsequent effect on CREB activity may differ [61, 63]. It may thus be assumed that combined treatment with AMA and FLU increases the levels of P-CREB to a greater extent in the cerebral cortex; hence, in our study we observed a more pronounced BDNF expression in the cerebral cortex than in the hippocampus after joint administration of either of those drugs. On the other hand, our previous study indicated that repeated co-administration of IMI and AMA evoked a more potent increase BDNF gene expression in the hippocampus than in cerebral cortex [45].

BDNF is not only a possible target of action of ADs, but produces an antidepressant-like effect, and may be one of the molecular mediators of ADs. For example, central administration of BDNF into the ventricles, hippocampus or midbrain of rats produces an antidepressant-like effect in animal models of depression [15, 54, 55]. In addition to its direct antidepressant effect, BDNF infusion also induces neurogenesis [38], which may directly or indirectly contribute to its antidepressant action during long-term administration [18]. Preclinical and clinical studies demonstrate that stress and depression lead to decreases in the total volume of the hippocampus, as well as to the atrophy and loss of neurons of this structure. In comparison with healthy individuals, a reduction in hippocampal cell volume has been observed in depressed humans in both magnetic resonance imaging and post-mortem studies [11, 18, 51, 52]. Furthermore, treatment with ADs has been shown to reverse or prevent such a decrease in hippocampal volume [69]. Many classes of ADs enhance both cell proliferation and neurogenesis in adult hippocampus, which requires a chronic (but not acute) mode of administration [11, 26]. These findings permit a conclusion that ADs stimulate not only secondary messenger systems leading to activation of transcription factors such as CREB, but also neurotrophic pathways, and thus increase hippocampal neurogenesis [27].

In addition, it has been reported that the antidepressant-induced up-regulation of BDNF signalling is thought to promote adaptive neuronal plasticity through effects on gene expression; however, no effector gene downstream of BDNF has been identified thus far. Local infusion of BDNF into the dentate gyrus induces long-term potentiation (BDNF-LTP) of synaptic transmission that requires up-regulation of the immediate early gene Arc. Recently, five genes (neuritin, Narp, TIEGI, Carp, and Arl4d) that are co-up-regulated with Arc during BDNF-LTP have been identified, and the expression of these genes in the dentate gyrus, hippocampus proper and prefrontal cortex after FLU treatment has been examined. That study showed that chronic, but not acute, FLU administration resulted in up-regulation of those BDNF-LTP-associated genes in a brain region-specific manner, and linked the chronic effect of AD treatment to molecular mechanisms underlying BDNF-induced synaptic plasticity [35].

In the light of all the above findings, our results seem to support the hypothesis that joint administration of ADs and AMA (an uncompetitive NMDA receptor antagonist) may evoke more effective antidepressant activity than treatment with AD alone. Moreover, the enhancement of neurotrophic system support and the associated augmentation of synaptic plasticity and function may constitute a basis for antidepressant efficacy and be a present and future focus in the search for a more rapid-acting and effective medication. It is also possible that these findings could account for the higher efficacy of combined ad-

ministration of AMA and an AD compared to an AD alone in treatment-resistant patients [41, 43, 60]. Thus, this may be an alternative strategy; however, this assumption needs to be further confirmed with clinical trials.

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