Recent studies indicate a role of the brain-derived neurotrophic factor (BDNF) in the pathophysiology of depression, as well as in the mechanism of action of antidepressant drugs (ADs). It has been shown that serum BDNF levels are decreased in depressed patients. Moreover, antidepressant treatment increases serum BDNF levels and it is positively correlated with medication response. In addition, repeated administration of ADs induces an increase in rat hippocampal or cortical BDNF gene expression. 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 the new AD mirtazapine (5 or 10 mg/kg) on BDNF mRNA level (the Northern blot) in rat hippocampus and cerebral cortex. Imipramine was used as a reference compound. The experiment was carried out on male Wistar rats. The tissue for biochemical assays was collected 24 h after the last doses of mirtazapine and imipramine. We also studied the effect of repeated mirtazapine on the action of the 5-HT2A receptor agonist (+)DOI in the behavioral test (head twitches induced by (+)DOI) in rats. The obtained results showed that, like imipramine (10 mg/kg), mirtazapine (10 mg/kg) increased BDNF gene expression in both the examined brain regions: in the hippocampus by 24.0 and 26.5%, in the cerebral cortex by 29.9 and 41.5%, respectively, compared with the vehicle-treated control. Neither mirtazapine nor imipramine administered repeatedly at a lower dose (5 mg/kg) significantly changed BDNF mRNA levels in the hippocampus and cerebral cortex. Repeated treatment with mirtazapine (10, but not 5 mg/kg) inhibited the behavioral syndrome induced by (+)DOI. This study provides first conclusive evidence that repeated mirtazapine administration increases BDNF mRNA levels; moreover, it indicates that the enhancement of BDNF gene expression may be essential for the clinical effect of mirtazapine.

Key words: repeated treatment, mirtazapine, imipramine, mRNA BDNF, 5-HT2A syndrome, rats
INTRODUCTION

Mirtazapine (Org 3770, Remeron) (1,2,3,4,10,14b-hexa-hydro-2-methyl-pyrazinol [2,1-alpyridol[2,3-c][2]benzazapine), is an antidepressant drug (AD) which enhances noradrenergic and serotonergic 5-HT_{1A} neurotransmission via an antagonistic action at central α_{2}-adrenergic autoreceptors and heteroreceptors and the blockade of 5-HT_{2} and 5-HT_{3} receptors (1 - 5). In contrast to imipramine or other tricyclic ADs, this drug does not inhibit noradrenaline (NA) or serotonin (5-HT) reuptake (2, 6).

Moreover, biochemical studies have shown that, like imipramine and other tricyclic ADs, mirtazapine administered repeatedly induces significant down-regulation of 5-HT_{2A} receptors and slightly reduces the density of β_{1}-adrenoreceptors in the frontal cortex of rats (7). When given repeatedly (10 mg/kg for 14 days), mirtazapine also increases the responsiveness of the α_{1}-adrenergic system (behavioral and biochemical changes) (8), but does not induce any adaptive changes in dopaminergic D_{2}/D_{3} receptors (9).

Despite more than 45 years of research, the mechanism of antidepressant action has not been fully elucidated so far. The majority of adaptive changes, proposed to be "responsible" for neurochemical antidepressant mechanisms, are not common for all antidepressant therapies (10 - 15). Hence a search for common alterations is still in progress. A number of recent findings point to a role of the brain-derived neurotrophic factor (BDNF) in depression and/or in the mechanism of action of ADs as one of their main targets. It has been shown that stress decreases the expression of BDNF in the hippocampus and other limbic brain regions (16), and that this effect may contribute to the recently demonstrated atrophy of stress-vulnerable neurons in the hippocampus (17). Moreover, studies using a postmortem human brain tissue indicated an increase in hippocampal BDNF immunoreactivity in subjects treated with ADs compared to untreated subjects (18). In addition, clinical studies showed low BDNF serum levels in patients with major depressive disorders compared to control subjects, moreover, they demonstrated that AD treatment increased serum BDNF levels in depressed patients up to the level found in healthy controls (19 - 23).

Furthermore, local infusion of BDNF into the midbrain and hippocampus caused antidepressant-like activity in two behavioral models of depression: the forced swimming and the learned helplessness (24 - 26). The above findings suggest that the increased expression of endogenous BDNF may have an antidepressant effect. In contrast, the loss of forebrain BDNF (in BDNF knockout mice) attenuated the action of the antidepressant, desipramine in the forced swimming test (27). Hence BDNF may exert an antidepressant effect - partly by regulating the noradrenergic and/or serotonergic systems (28).

In addition, recent studies demonstrated that repeated (but not acute) administration of different ADs, including tricyclic ADs and serotonin selective reuptake inhibitors (SSRI), increased BDNF and trkB (a receptor for BDNF)
mRNA in the hippocampus (29 - 35), whereas, electroconvulsive shock (ECS) and tranylcypromine (a monoamine oxidase inhibitor (MAOI)) induced a similar effect in the hippocampus and cerebral cortex (29).

Since the most potent effect of ADs on BDNF gene expression was found after prolonged treatment, the present study investigated the influence of repeated treatment (twice daily for 14 days) with mirtazapine (5 or 10 mg/kg) on BDNF mRNA level in rat hippocampus and cerebral cortex (whose effect on BDNF expression had not been studied before). Measurements were performed 24 h after the last oral administration. This chosen time point was considered important since a recent study has shown that long term systemic injection of some ADs result in a bi-phasic change in BDNF mRNA levels, whereby there was a decrease at 4 h and an increase at 24 h after the last injection (31). To control experimental conditions, we also examined the effect of the classic AD imipramine. In addition, the effect of repeated mirtazapine on the action of the 5-HT\textsubscript{2A} agonist (±)DOI was studied using a behavioral test in rats.

MATERIALS AND METHODS

Animals and drug treatment

The experiments were carried out on male Wistar rats ca. 80 days old, weighing 220-230 g at the beginning of the study. After 14 days of repeated drug administration, the weight of the animals increased up to 270-300 g. The animals had free access to food and water during the experiment and were kept at a constant room temperature (22 ± 1°C) on a 12/12-hour light/dark cycle (the light on at 7 a.m.). Mirtazapine (5 and 10 mg/kg) was suspended in a 1% aqueous solution of Tween 80, or imipramine (5 or 10 mg/kg) was dissolved in distilled water, and either drug was administered repeatedly (twice daily p.o. for 14 days) in a volume of 2 ml/kg. Control animals received vehicle (1% aqueous solution of Tween 80 and distilled water for mirtazapine and imipramine, respectively) according to the same schedule. Twenty-four hours after the last treatment, the rats were decapitated, their hippocampi and frontal cortices were dissected and immediately frozen on dry ice and stored until they were used for biochemical experiments. The behavioral syndrome (head twitches induced by (±)DOI, a 5-HT\textsubscript{2A} receptor agonist) was measured 2 or 24 h after the last dose of mirtazapine (or after its single administration). Drug treatments were randomly tested and the animals were used for experiments only once. The experimental protocols were approved by the local Ethics Committee and complied with the guidelines of the responsible agency of the Institute of Pharmacology.

Drugs

Imipramine hydrochloride (Sigma, USA; IMI), (±)-1-(4-jodo-2,5-dimethoxy-phenyl)-2-aminopropane hydrochloride (Research Biochemicals Int., USA; (±)DOI) and mirtazapine (1,2,3,4,10,14b-hexa-hydro-2-methyl-pyrazinol[2,1-alpyridol[2,3-c][2]benzazapine, Org 3770, Remeron) (Organon, The Netherlands; MIR) were used for the present study.

Determination of BDNF mRNA

The procedure for determination of BDNF mRNA levels was performed according to Legutko et al. (36) and Nowak et al. (37). Briefly, total RNA was extracted by a chaotropic lysis (TRIzol Reagent, Life Technologies) following the manufacturer's protocol. The Northern blot analysis was
performed with ~7 µg of total RNA, separated on a 1% denaturing agarose-formaldehyde gel, subsequently transferred to a nylon membrane (Nytran, Schleicher and Schuell) and immobilized by ultraviolet (UV) radiation. A probe for rat BDNF was generated by a polymerase chain reaction (PCR) from cDNA, 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 pCRII TA cloning vector. The insert cut with enzyme EcoRI was random 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 twice for 30 min in a saline-sodium citrate (SSC) buffer/0.1% sodium dodecyl sulfate (SDS) at a room temperature, and for 30 min in 0.1 x SSC/0.1% SDS at 55 ° and exposed. The same filters, stripped off the BDNF probe (washed three times in a 0.1 x SSC/0.1% SDS at 100° for 10 min), were re-hybridized for a β-action cDNA probe (Clontech) to normalize RNA loading. Northern blots were quantified using digitalized autoradiographs (Phosphor-Imager, Image 4.0 Fuji). Each group consisted of 5-6 rats.

**Head twitches induced by (±)DOI**

The separate groups of rats were used for the Northern blot analyses and the behavioral measurement. (±)DOI (2.5 mg/kg) was given 2 or 24 h after the last dose of mirtazapine (or after its single administration). Head twitches reaction were recorded immediately after (±)DOI administration, and the recording was continued for 30 min. Each group consisted of 8 rats.

**Statistical analysis**

The data were evaluated by 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 mirtazapine and imipramine on BDNF mRNA level in rat hippocampus and cerebral cortex is shown in Fig. 1. In the hippocampus, repeated treatment with mirtazapine or imipramine at a higher dose (10 mg/kg) significantly elevated BDNF mRNA levels compared to the vehicle-treated control (by 24.0 and 26.5%, respectively). Only a slight increase was observed after a lower dose (5 mg/kg) of either antidepressant (by 13.4 and 5.1%, respectively), but the difference was not statistically significant (ANOVA; F(4,24)=4.56, p<0.001; Fig. 1A). Similarly, in the cerebral cortex of rats, mirtazapine (10 mg/kg) - like imipramine (10 mg/kg) - significantly increased BDNF mRNA levels compared to the vehicle-treated control (by 29.9 - 41.5%). In contrast, neither mirtazapine nor imipramine administered repeatedly at the lower dose (5 mg/kg) significantly change BDNF mRNA expression in the cerebral cortex (ANOVA; F(4, 30)=15.74, p<0.001; Fig. 1B).

**Head twitches induced by (±)DOI**

Mirtazapine (5 mg/kg) given in a single dose did not change significantly the head twitches induced by 5-HT\(_{2A}\) agonist, (±)DOI (2.5 mg/kg). The higher dose
of this drug (10 mg/kg) reduced the head twitches induced by (±)DOI only at 2 h, but not at 24 h after single administration, (ANOVA; F(4,35)=5.00, p<0.01; Fig. 2A). Repeated treatment with mirtazapine (10 mg/kg, but not 5 mg/kg) inhibited the behavioral syndrome induced by (±)DOI (measured at 2 and 24 h after the last dose of the drug) (ANOVA; F(4,35)=30.96, p<0.001; Fig. 2B).

**DISCUSSION**

The present study was aimed at investigating the effect of repeated mirtazapine administered (twice daily for 14 days) on BDNF mRNA levels.
(measured 24 h after the last drug administration) in rat hippocampus and cerebral cortex. The obtained results indicate that mirtazapine given repeatedly increases BDNF gene expression in both the examined brain regions (hippocampus and cerebral cortex). The effect of mirtazapine in that test was similar to that produced by the classic AD imipramine or MAOI, tranylcypromine and ECS (29). Moreover, it suggested that ADs that enhanced noradrenergic and serotonergic neurotransmission increased BDNF expression not only in the hippocampus, but also in the cerebral cortex. In addition, the present study indicated that repeated mirtazapine administration inhibited 5-HT₂A neurotransmission in the behavioral test, that effect being in line with the previously demonstrated decrease in the

Fig. 2. The effect of mirtazapine (MIR) on (±)DOI-induced head twitches in rats. MIR (5 or 10 mg/kg) was given in a single dose or repeatedly (twice daily for 14 days). The test was carried out 2 or 24 h after a single dose (A) or the last dose (B) of MIR (or vehicle). (±)DOI (2.5 mg/kg) was injected directly before the test. The results are presented as the mean ± SEM of 8 animals/group. The data were statistically evaluated by ANOVA, followed by individual comparisons using Dunnett's test. *p<0.001 vs. (±)DOI group.
density of 5-HT<sub>2A</sub> receptors, observed in biochemical experiments (7). Similar behavioral and biochemical effects on 5-HT<sub>2A</sub> receptors were produced by imipramine (38) and other ADs studied previously (39, 40).

An initial study of the effect of several ADs on BDNF gene expression demonstrated its increase 2-3 h after the last drug administration according to repeated (21 days), but not acute (one day) schedule (29). The effect of desipramine, sertraline and mianserine could be seen in the hippocampus, while ECS and tranylcypromine induced a similar effect in the hippocampus and cerebral cortex (29). In contrast, repeated administration of such nonantidepressant psychotropic drugs as morphine, cocaine or haloperidol did not increase the levels of BDNF mRNA in the hippocampus and cerebral cortex (29). Furthermore, repeated administration of ECS or ADs completely blocked the down-regulation of BDNF mRNA in the hippocampus in response to restraint stress (29), which suggests that the enhanced induction and prolonged expression of BDNF in response to chronic ECS and AD treatments may promote neuronal survival and protect neurons from the damaging effects of stress. A more recent paper by Coppell et al. (31) showed that some ADs, that enhanced serotonergic neurotransmission (fluoxetine, paroxetine sertraline or tranylcypromine), but not desipramine, maprotyline or mianserin, induced an increase in the hippocampal (especially the dentate gyrus) BDNF expression 24 h after repeated treatment (twice daily for 14 days), but decreased that expression at 4 h after acute or repeated treatment. These above data suggest that the effect of ADs on BDNF gene expression may be more complex and less widespread across treatments than was previously believed; they also show that drugs interacting with the central 5-HT system alter BDNF gene expression in the hippocampus, this effect being biphasic over a 24 h post-drug period, though. Administration of NA reuptake inhibitors (desipramine and maprotyline) or the atypical AD mianserin had no effect on BDNF mRNA levels at either single (4 h post drug) or repeated (4 and 24 h post drug) treatment (31). In contrast, a significant increase caused by the specific reuptake inhibitor NA (desipramine) on BDNF expression in the hippocampus was reported by some authors (29, 32, 35, 41), but not by Coppell et al. (31) and Russo-Neustadt et al. (42). In addition, BDNF protein levels were unaltered after the latter drug (43), but were elevated (3-fold) extracellular NA in the hippocampus and frontal cortex (44, 45). In marked contrast to the findings that desipramine increases BDNF mRNA expression (29, 32, 35, 41), is the observation that denervation of NA axons increases BDNF mRNA expression in the hippocampus (46), which suggests that NA tonically inhibits BDNF mRNA expression. Thus the noradrenergic regulation of BDNF mRNA and protein expression seems to be a complex phenomenon and may explain the discrepancies between studies.

Moreover, the study of Nibuya et al. (30) also demonstrated that repeated, but not acute, administration of several different classes of ADs, as well as ECS application, increased the expression of cAMP response element binding protein
(CREB) mRNA in the hippocampus, and suggested that transcription factor was a common intracellular target of ADs. The above results also support the hypothesis that 5-HT- and NA receptor-coupled intracellular cascades which lead to CREB regulation are activated, not down-regulated, by repeated AD treatments. This observation suggests that although levels of certain 5-HT and NA receptors (e.g., 5-HT$_{2A}$, 5-HT$_{7}$ and $\beta$-adrenergic receptors) may be reduced by repeated AD treatments, an increase occurs in the functional output of these receptor-coupled intracellular pathways. This is not surprising, since the density and function of these receptors are reduced, but not completely eliminated, by repeated AD treatments (47). Activation of the remaining 5-HT and NA receptors may be sufficient to stimulate intracellular signal transduction pathways. It is noteworthy to point out that the up-regulation of CREB is a common effect of repeated AD treatment that may lead to regulation of specific target genes such as BDNF and trkB, and to a long-term impact of these treatments on brain functions.

In conclusion, our results indicate that, like imipramine, mirtazapine increased BDNF gene expression in the hippocampus and cerebral cortex. These finding provide strong evidence that the increased expression of BDNF is a downstream effect of increased 5-HT/NA neurotransmission, and that it may be responsible for the therapeutic effect of such ADs as mirtazapine or imipramine.

Acknowledgments: The authors are grateful to Organon for the generous gift of mirtazapine. We would also like to thank Ms. E. Smolak, M.A., for the linguistic correction of the paper.

REFERENCES


Received: August 8, 2005
Accepted: November 4, 2005

Author’s address: Dr Zofia Rogóź, Department of Pharmacology, Institute of Pharmacology, Polish Academy of Sciences, 12 Smętna Street, 31-343 Kraków, Poland, fax: +48 12 63 74 500; tel: +48 12 66 23 279. E-mail: rogoz@if-pan.krakow.pl