Mirtazapine (MIR) is an antidepressant which enhances noradrenergic and serotonergic 5-HT$_{1A}$ neurotransmission via antagonism of central $\alpha_2$-adrenergic autoreceptors and heteroreceptors. The drugs does not inhibit noradrenaline and serotonin reuptake but blocks the 5-HT$_{4}$ and 5-HT$_{3}$ receptors and has high affinity only for central and peripheral histamine H$_1$ receptors. The present study was aimed at determining whether repeated MIR treatment induced adaptive changes in the $\alpha_1$-adrenergic receptors, similar to those reported by us early for tricyclic antidepressants, The experiments were carried out on male mice and rats. MIR was administered at a dose of 10 mg/kg once or repeatedly (twice daily for 14 days). The obtained results showed that MIR administrated repeatedly potentiated the methoxamine- induced exploratory hyperactivity in rats and clonidine-induced aggressiveness in mice, those effects being mediated by $\alpha_1$-adrenergic receptors. MIR given repeatedly (but not acutely) increased the binding ($B_{\text{max}}$) of [H]prazosin to $\alpha_1$-adrenergic receptors in cerebral cortex, however, the ability of the $\alpha_1$-adrenoceptor agonist phenylephrine to compete for these sites was not significantly changed. The above results indicate that repeated MIR administration increases the responsiveness of $\alpha_1$-adrenergic system (behavioural and biochemical changes), as tricyclics do. However, the question whether the increased functional responsiveness found in the present study is important for the clinical antidepressant efficacy, remains open.

**INTRODUCTION**

Mirtazapine (Org 3770)(1,2,3,4,10,14b-hexa-hydro-2-methyl-pyrazino[2,1-alpyridol[2,3-c][2]benzazepine, MIR), the 6-aza-analogue of mianserin, is an antidepressant drug which enhances noradrenergic and serotonergic 5-HT$_{1A}$ neurotransmission via antagonism of central $\alpha_2$-adrenergic autoreceptors and
heteroreceptors and blockade of 5-HT$_2$ and 5-HT$_3$ receptors (1, 2, 3, 4, 5). MIR has no affinity for dopaminergic, cholinergic and muscarinic receptors, but has only high affinity for histamine H$_1$ receptors (1, 6). The drug does not inhibit noradrenaline or serotonin uptake (1, 6).

The antidepressant activity of MIR in the clinic is comparable to that of typical tricyclics such as amitriptyline and clomipramine (7, 8).

Biochemical and behavioural studies in animals have shown that MIR administered repeatedly induced significant down-regulation of 5-HT$_{2A}$ receptors and it reduced slightly the density of β$_1$-adrenoreceptors in the frontal cortex of rats (9).

Our earlier studies show that antidepressant drugs (ADs) administered repeatedly (but not in a single dose) increase, among others, responsiveness of the α$_1$-adrenergic system (sensitivity of postsynaptic α$_1$-adrenergic receptors). The measure of the above activity is potentiation of the behavioural hyperexploration evoked by α$_1$-adrenergic agonists (phenylephrine, methoxamine), as well as of clonidine-induced aggressiveness (10 - 12), since pro-aggressive effect of clonidine (in a high dose of the drug, i.e. 20 mg/kg) results from the stimulation of postsynaptic α$_1$-adrenergic receptors (10, 13). Moreover, ADs administered repeatedly increase the binding to α$_1$-adrenergic receptors in different brain regions, in particular the affinity of these receptors for their agonists (i.e. also to noradrenaline, the endogenous neuromediator) (14, 15). The above effects have been described for various ADs (tricyclics, selective serotonin reuptake inhibitors (SSRIs), MAO inhibitors, mianserin) (10 - 12).

The present paper was aimed at determining whether MIR, which enhances noradrenergic and serotonergic 5-HT$_{1A}$ neurotransmission via antagonism of central α$_2$-adrenergic autoreceptors and heteroreceptors, evokes, when given repeatedly, adaptive changes in the α$_1$-adrenergic system, similar to those produced by tricyclic drugs. To this end, we administered MIR for two weeks (twice daily) i.e. for a period and dosage generally accepted in the treatment with tricyclics and studied its effects on the response to the agonist for α$_1$-adrenergic system in the following tests:
1. α$_1$-adrenergic agonists: the methoxamine-induced hyperexploratory behaviour in rats;
2. the clonidine-induced aggressiveness in mice.

We also studied the effect of repeated MIR administration on the binding of [$^3$H]prazosin to α$_1$-adrenergic receptors in the rat cortex.

**MATERIALS AND METHODS**

**Animals**

The experiments were carried out on rats (male Wistar, healthy, ca. 80 days old, weighing 220-230 g; after 14 days of repeated drug administration the weight of animals increased up to 270-300 g) and mice (male Albino-Swiss, ca. 50 days old, weighing 25-30 g). The animals had
free access to food and water before the experiment and were kept at a constant room temperature
(22 ± 1°C), under a 12 hour light/dark cycle (light on at 7 a.m.). Experimental protocols were
approved by the local Ethics Committee and met guidelines of the responsible Agency of the
Institute of Pharmacology.

Drugs

Clonidine hydrochloride (CLO; Research Biochemicals Int.), methoxamine hydrochloride (MET,
Vasoxine amp., Wellcome), mirtazapine (MIR; Organon), pentobarbital (Vetbutal amp., Biowet),
phenylephrine hydrochloride (Research Biochemicals Int.), [3H]prazosin (NEN Du Pont, UK).

Drug administration

MIR (10 mg/kg) was suspended in a 1% aqueous solution of Tween 80 and was administered
perorally (p.o.) with a stomach tube, once or repeatedly (twice daily for 14 days). All animals
received treatment twice daily for 14 days. Drug was administered at 8-9 o’clock a.m. and 8-9
o’clock p.m. Control animals received vehicle for the whole period of time. Repeatedly treated
animals received the appropriate drug, and animals treated acutely received vehicle for 13 days,
and on the day 14, they received the appropriate drug, so all groups of animals were handled in the
same manner. Using this experimental paradigm we avoided the effect of a single intragastric
intubation which inevitably, as a stressful event for an animal, may mask or change the actual
effect of acute administration of the studied drug. All groups of animals, treated acutely or repeatedly,
were taken for the behavioural experiment or decapitated for biochemical assay at the same time.

The behavioural experiments were carried out 2 or 72 h after a single (acute treatment) or last
dose (repeated treatment) of MIR. For biochemical experiments the rats were sacrificed 2 or 72 h
after a single (acute treatment) or the last dose (repeated treatment) of MIR. The tissue (cortex)
was dissected out and frozen on dry ice, and stored until used for binding experiments.

Statistical analysis

The behavioural data were evaluated by one-way analysis of variance (ANOVA) followed,
when appropriate, by individual comparisons with the control using Dunnett’s test. The binding
results were statistically assessed by ANOVA, intergroup differences were analysed by Duncan’s
multiple range test.

Behavioural studies

Exploratory behaviour induced by methoxamine in rats

For experiments with methoxamine, the rats were operated under pentobarbital anaesthesia (30
mg/kg i.p.). They were implanted chronically and unilaterally with stainless steel guide cannulae
9.00 mm long (0.4 mm o.d.), according to the method described by Kolasiewicz and Maj (16).
After a 4-day postoperative period, the animals were administered MIR (10 mg/kg p.o.) twice
daily for 14 days. Control animals were given vehicle. Methoxamine were injected at a dose of 25
µg/5 µl into the brain lateral ventricle (at 90 min after the last dose of MIR and 30 min before the
test), using an inner injection cannula (11.6-14.6 mm long; 0.3 mm o.d.). The tip of injection cannula was aimed at the lateral ventricle (AP (-) 0.4-0.8, L 1.1-1.7,) using stereotaxic coordinates (17). Injection of the volume of 5 µl lasted 2 min. The inner cannula was withdrawn 1 min after the termination of the injection. Control animals (operated) were treated with appropriate volume of the solvent. Exploratory activity was assessed in the elevated open field test by a method used previously by Maj and Rogóz (18). The black circular elevated platform (without walls, 1 m in diameter, divided into six symmetrical sectors, elevated 50 cm above the floor) was used. During the experiment, the laboratory room was dark and only the centre of the open field was illuminated with 75 W bulb, hung directly above it, 75 cm high. The animals were placed in the open field and their exploratory behaviour, i.e. the time of walking, number of crossings (ambulation), episodes of peeping outside the edge of the arena and rearing, was assessed for 3 min. After completion of the experiments, rats were anesthetized with 45 mg/kg pentobarbital, perfused through the heart with 4% paraformaldehyde, and decapitated. The brains were cut into 50 µm sections and the location of all the injection cannulae tips was determined histologically. Only those animals with histologically confirmed injection sites were used for the data analysis. Each group consisted of 8 rats.

Clonidine-induced aggressive behaviour in mice

Two or 72 hours after the last dose of MIR (or after its single administration), CLO was injected i.p. at a dose of 20 mg/kg. Immediately thereafter, groups of 4 mice each were placed together in plexiglass cages (20 × 15 cm) (13) and were observed for 1 h. Aggression was expressed as the number of biting attacks among 4 mice within 1 h. Each group consisted of 32 mice.

Biochemical studies

α₁-Adrenergic receptor binding in the rat brain cortex

The experiment was carried out according to the method used previously (Maj et al. (19, 20). For [³H]prazosin (specific activity: 19.5 Ci / nmol) binding studies, the tissue was homogenized for 15 s in 20 vol. (w/v) of an ice-cold Tris–HCl buffer (50 mM, pH 7.4) using Ultra Turrax homogenizer. The nonspecific binding was defined in the presence of 10 µM regitine. The homogenates were centrifuged at 25,000 x g for 10 min. That step was repeated twice. Final pellets were resuspended in 170 vol. (w/v) of a Tris–HCl buffer (50 mM, pH 7.4). Saturation isotherms were generated using eight concentrations (0.01- 2nM) of [³H]prazosin. The bound ligand was separated by vacuum filtration through Whatman GF/C filters and was washed three times with 5 ml of ice-cold Tris–HCl buffer. Radioactivity was measured in Beekman LS 6500 scintillation counter. All assays were performed in duplicate. The data were analyzed using iterative fitting routines (Graph PAD Prism 2.0). Each group consisted of 6-8 rats.

Phenylephrine competition for the [³H]prazosin binding in the rat brain cortex

The experiment was carried out according to the method used previously (Maj et al. (19, 20). The affinity of α₁-adrenergic receptors for an agonist was estimated by studying the ability of various concentrations of phenylephrine (0.1 nM -1 mM) to compete for [³H]prazosin binding sites. To a volume of 1.7 ml of tissue suspension 200 µl of phenylephrine and 100 µl of [³H]prazosin (final concentration: 0.5 nM) were added. Afterwards, the samples were incubated at 25° C for 25 min, followed by a 10 min ice-cold bath. Finally, a total incubation volume of 2 ml was poured
over glass filters (Whatman GF/C) and rinsed three times with 5 ml of an ice-cold Tris-HCl buffer. All assays were performed in duplicate. The data were analyzed using iterative fitting routines (Graph PAD Prism 2.0). Each group consisted of 6-8 rats.

RESULTS

Behavioural studies

Methoxamine-induced exploratory hyperactivity in rats

Methoxamine (25 µg/5 µl) given intraventricularly increased exploratory behaviour in the open field test (time of walking, ambulation, peeping and rearing). MIR in a single dose (10 mg/kg) neither affected the exploratory activity in normal rats (Table 1) nor changed the action of methoxamine (Table 1). MIR (10 mg/kg) administered repeatedly did not affect the exploratory activity in the open field test (Table 1). Repeated treatment with MIR, 10 mg/kg, enhanced the effect induced by methoxamine in the open field test; it prolonged the time of walking, increased the number of ambulations (measured 2 and 72 h) and rearing and peeping episodes (measured only 2 h) after the last dose of the drug (Table 1).

Clonidine-induced aggressive behaviour in mice

Neither MIR, given acutely or repeatedly in the dose used, evoked any aggressive behaviour in mice.

A single dose (10 mg/kg) of MIR did not change the aggression induced by CLO (20 mg/kg) (Table 2). Repeated treatment with MIR, 10 mg/kg enhanced the effect induced by CLO at 2 and 72 h after the last dose MIR (Table 2).

Table 1. Effects of single and repeated treatment with mirtazapine (MIR) on the exploratory behaviour induced by methoxamine (MET) in rats. MET, 25µg / 5µg was injected into brain lateral ventricle 30 min before the test. The test was carried out at 2 or 72 h after the last dose of MIR. Data represent mean ± SEM, n=8. The statistical significance was assessed using ANOVA, followed, when appropriate, by the Dunnett’s test. *p<0.05, **p<0.001 vs vehicle receiving group, #p<0.05, ##p<0.001 vs MET receiving groups.

<table>
<thead>
<tr>
<th>Compounds (mg/kg)</th>
<th>Acute treatment (mean ± SEM)</th>
<th>Repeated treatment (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time of walking</td>
<td>Ambulation</td>
</tr>
<tr>
<td>Vehicle</td>
<td>35.3 ± 2.0</td>
<td>12.1 ± 1.2</td>
</tr>
<tr>
<td>MET</td>
<td>75.5 ± 2.5**</td>
<td>23.8 ± 1.7**</td>
</tr>
<tr>
<td>MIR10, 2h</td>
<td>36.3 ± 1.8</td>
<td>12.6 ± 1.2</td>
</tr>
<tr>
<td>MIR10, 72h</td>
<td>33.3 ± 0.8</td>
<td>10.5 ± 0.9</td>
</tr>
<tr>
<td>MIR10 + MET, 2h</td>
<td>70.5 ± 3.2</td>
<td>19.4 ± 2.1</td>
</tr>
<tr>
<td>MIR10 + MET, 72h</td>
<td>70.6 ± 5.0</td>
<td>24.9 ± 3.9</td>
</tr>
</tbody>
</table>
Table 2. Effect of single and repeated treatment with mirtazapine (MIR) on the clonidine (CLO)-induced aggressive behaviour in mice. MIR (10 mg/kg p.o.) was given in a single dose or repeatedly (twice daily for 14 days), CLO (20 mg/kg i.p.) at 2 or 72 h after the last injection of MIR. Aggression is expressed as the number of biting attacks among 4 mice within 1h after CLO-administration. Data represents mean ± SEM, n=8. The statistical significance was assessed using ANOVA, followed when appropriate, by the Dunnett’s test. *p<0.05 vs CLO-receiving group.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Number of biting attacks</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLO</td>
<td>62.5 ± 8.9</td>
</tr>
<tr>
<td>MIR 10, single + CLO, 2h</td>
<td>59.4 ± 6.9</td>
</tr>
<tr>
<td>MIR 10, single + CLO, 72h</td>
<td>62.8 ± 3.5</td>
</tr>
<tr>
<td>MIR 10, repeated + CLO, 2h</td>
<td>89.0 ± 6.3*</td>
</tr>
<tr>
<td>MIR 10, repeated + CLO, 72h</td>
<td>80.9 ± 2.3*</td>
</tr>
</tbody>
</table>

Table 3. Effects of single and repeated treatment with mirtazapine (MIR) on the competition of phenylephrine for \[^{3}H\] prazosin binding sites in the rat brain cortex. MIR (10 mg/kg p.o.) was given in a single dose or repeatedly (twice daily for 14 days). The tissue for biochemical measurements was taken out at 2 or 72 h after single or last dose of the drug. Data represents mean ± SEM, n = 6-8. The statistical significance was assessed using ANOVA, followed, when appropriate, by Dunnett’s test. *p<0.05 vs vehicle-receiving group.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>B(_{\text{max}}) [fmol/mg protein]</th>
<th>K(_d) [nM]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>8.28 ± 0.9</td>
<td>0.41 ± 0.11</td>
</tr>
<tr>
<td>MIR 10, single 2h</td>
<td>10.52 ± 1.2</td>
<td>0.43 ± 0.12</td>
</tr>
<tr>
<td>MIR 10, single 72h</td>
<td>8.81 ± 0.8</td>
<td>0.45 ± 0.14</td>
</tr>
<tr>
<td>MIR 10, repeated 2h</td>
<td>10.56 ± 0.9</td>
<td>0.46 ± 0.08</td>
</tr>
<tr>
<td>MIR 10, repeated 72h</td>
<td>13.80 ± 1.8*</td>
<td>0.55 ± 0.06</td>
</tr>
</tbody>
</table>

Table 4. Effects of single and repeated treatment with mirtazapine (MIR) on the binding of \[^{3}H\] prazosin to \(\alpha_1\)-adrenergic receptors in the rat brain cortex. MIR, (10 mg/kg p.o.) was given in a single dose or repeatedly (twice daily for 14 days). Data represents mean ± SEM, n = 6-8. The statistical significance was assessed using ANOVA.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>K(_i) [(\mu)M]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>34.7 ± 1.8</td>
</tr>
<tr>
<td>MIR 10, single 2h</td>
<td>41.1 ± 0.5</td>
</tr>
<tr>
<td>MIR 10, single 72h</td>
<td>35.4 ± 1.9</td>
</tr>
<tr>
<td>MIR 10, repeated 2h</td>
<td>38.5 ± 1.2</td>
</tr>
<tr>
<td>MIR 10, repeated 72h</td>
<td>37.1 ± 0.4</td>
</tr>
</tbody>
</table>
Biochemical studies

\(\alpha_1\)-Adrenergic receptor binding in the rat brain cortex

MIR (10 mg/kg) administered in a single dose did not change the density (\(B_{max}\)) of \([^3H]\text{prazosin}\) binding sites (*Table 3*), or the binding affinity (\(K_D\)) (*Table 3*) in the cerebral cortex, measured 2 or 72 h after a single dose of the drug. Repeated treatment with MIR (10 mg/kg) increased the density (\(B_{max}\)) of \([^3H]\text{prazosin}\) binding sites (only at 72 h after the last dose) (*Table 3*), but not the affinity in the cerebral cortex (*Table 3*). Phenylephrine competition for the \([^3H]\text{prazosin}\) binding in the rat brain cortex

Competition studies showed that administration of MIR, 10 mg/kg, given in single doses or repeatedly, did not change the ability of phenylephrine to displace \([^3H]\text{prazosin}\) from cortical \(\alpha_1\)-adrenoceptors, since the \(K_i\) value was not changed (*Table 4*). The effect was measured at 2 or 72 h after a single or the last dose (repeated treatment) of the drug.

DISCUSSION

The aim of the present study was to investigate the effect of the new antidepressant MIR, administered repeatedly (14 days), on the \(\alpha_1\)-adrenergic system. The obtained results indicate that MIR, given repeatedly (but not acutely), potentiated the methoxamine-induced exploratory hyperactivity in the open field test in rats. Similarly, the clonidine-induced aggression in mice was enhanced by repeated treatment with MIR. This aggressiveness results from the stimulation of \(\alpha_1\)-adrenergic receptors (10, 13). The results indicate that MIR given repeatedly evokes hyperresponsiveness of \(\alpha_1\)-adrenoceptors. Such an activity was observed earlier following repeated administration of tricyclic antidepressants (10, 11, 12).

The biochemical data show that MIR administered acutely did not change the binding parameters of \([^3H]\text{prazosin}\) to \(\alpha_1\)-adrenergic receptors in the rat brain cortex. Repeated treatment with MIR at 72 h after the last dose increased the density (\(B_{max}\)) of \(\alpha_1\)-adrenergic receptors in this brain structure.

On the other hand, when the competition of phenylephrine (\(\alpha_1\)-adrenoceptor agonist) for \([^3H]\text{prazosin}\) binding sites (i.e. \([^3H]\text{prazosin}\) displacement by phenylephrine) was studied, no significant changes after acute or repeated treatment with MIR was observed.

These results indicate that the increased responsiveness of \(\alpha_1\)-adrenergic receptors observed in the behavioral experiments (i.e. increased methoxamine-induced exploratory hyperactivity) might result from the increased number of \(\alpha_1\)-adrenergic receptors, but not from the affinity of these receptors for phenylephrine, which was not changed after the repeated treatment with MIR. However, we did not observe any
significant changes in the density of $\alpha_1$-adrenergic receptors at 2 h after last dose of repeatedly administered MIR, although the behavioral responsiveness was increased also at that time. It is difficult to explain this difference. At 2 h after the administration MIR is still present in the rat brain (4) in the vicinity of $\alpha_1$-adrenergic receptors, what might be a cause of the lack of effects observed in the binding studies, just because of physical interaction with the $[^3H]$prazosin used as a ligand or because of alterations in the neuronal membrane fluidity.

On the other hand, parallel changes in the response of $\alpha_1$-adrenergic receptors both at behavioral as well as biochemical level were observed at 72 h after the repeated administration of MIR. Similar parallelism was observed in the recent studies of the effects of mianserin. Repeated administration of mianserin, structural analog of MIR, which – in contrast to MIR – has a high affinity for monoaminergic ($\alpha_1$-adrenergic and $H_1$ histaminergic receptors), induced the behavioral as well as biochemical up-regulation of $\alpha_1$-adrenergic receptors at 24 h after the last dose (11, 21, 26).

In the light of such interpretation it seems that the affinity of MIR and mianserin for $H_1$-histaminergic receptors is of no importance for the differences in the changes in density of $\alpha_1$-adrenergic receptors after repeated administration of these drugs. We think that it is rather the presence or absence of the drug in the vicinity of receptors that matters.

Our earlier investigations showed that imipramine and amitriptyline as well as citalopram or mianserin, given repeatedly, increased the binding ($B_{\text{max}}$) to $\alpha_1$-adrenoceptors in the cerebral cortex of the rat when $[^3H]$prazosin was used as a ligand (21). The increased binding to $\alpha_1$-adrenergic receptors in the cortex and the other brain structures after repeated administration of ADs was confirmed by several authors (14, 22, 23). Nowak and Przegalinski (14) have also reported an increase of binding to $\alpha_1$-adrenoceptors in the cerebral cortex. However, the lack of an increase in density of $\alpha_1$-adrenergic receptors after repeated administration of antidepressant drugs was also demonstrated (12, 24, 25). On the other hand, using a different approach, i.e. the method of $[^3H]$prazosin displacement by phenylephrine, Menkes et al. (15) showed that amitriptyline, desipramine and iprindole increased the affinity of $\alpha_1$-adrenergic receptors for their agonists. A similar effect was demonstrated for imipramine, amitriptyline, citalopram, mianserin, (26) as well as milnacipran, venlafaxine or tianeptine (20, 26-28).

There are also electrophysiological studies concerning the effects of antidepressant drugs on the $\alpha_1$-adrenergic receptor-mediated responses. Most of these experiments have been done on hippocampus. The obtained data suggest an enhancement of $\alpha_1$-adrenergic responses in hippocampus following long-term antidepressant treatments. A two-week administration of imipramine and mianserin increased the suppressant effect of phenylephrine on the firing rate of CA1 hippocampal neurons in vitro (29). However, treatment with MAOIs and desipramine did not change the efficacy of the electrical stimulation (at 1Hz) of the locus coeruleus pathway to suppress the firing
activity of \( \text{CA}_3 \) pyramidal neurons, an effect which is mediated through \( \alpha_1 \)-adrenoceptors (see: Mongeau et al. (30)). The results of Bijak (29) appear consistent with the enhancement of affinity and sensitivity of brain \( \alpha_1 \)-adrenoceptors following long term antidepressant drugs that Aghajanian and co-workers found in other regions of the brain (31).

The above described results suggest a mechanism whereby \( \alpha_1 \)-adrenoceptors become functionally supersensitive with antidepressant treatment (increase in either their number or their affinity for an agonist). This finding may have relevance for the noradrenergic hypothesis of depression which posits a functional deficit of noradrenaline in this disease. On the other hand, clinical neuroendocrine studies have indicated \( \alpha_1 \)-adrenergic subsensitivity in certain depressives (32), what may indicate that regulation of \( \alpha_1 \)-adrenergic receptors might be related also to the therapeutic action of MIR in man, besides other possible mechanisms.

However, the overall antidepressant activity of MIR is believed to result from combined noradrenergic and serotonergic effects, in particular, its pharmacological profile is characterised by antagonism of central \( \alpha_2 \)-adrenergic autoreceptors and heteroreceptors, as well as by blockade postsynaptic of serotonin 5-HT\(_2\) and 5-HT\(_3\) receptors (4). Studies in animals models have shown that the serotonergic firing rate is increased soon after the administration of MIR (in contrast to the selective serotonin reuptake inhibitors [SSRIs], which reduce the firing rate soon after administration), what suggest that the drug may have a faster onset of action than the SSRIs (4). Furthermore, the antagonism of 5-HT\(_2\) and 5-HT\(_3\) receptors by MIR indicates that, therapeutically, the drug may have anxiolytic effect, improves sleep and being devoid of typical adverse effects of SSRI, such as agitation, restlessness, sexual dysfunction, nausea, vomiting and headache (4). Moreover, clinical studies revealed the high therapeutic efficacy of MIR in treating pathological craving for alcohol and alcohol-related affective disorders. The drug had also anxiolytic, hypnotic and vegeto-stabilizing effects. On this basis, MIR might be recommended as an effective and harmless medication to be included in the complex therapeutic programs for treating alcoholic patients (33, 34). The behavioural studies also seem to indicate possible importance of serotonin 5-HT\(_{2A/2C}\) receptor antagonists in the therapy of cocaine addiction (35). It is tempting to suggest that similar mechanism may underlie the effectiveness of MIR in treating alcohol craving. Two weeks MIR administration in rats caused significant down – regulation of 5-HT\(_2\) receptors in the frontal cortex, however that drug had no direct effects on 5-HT\(_{1A}\) receptor function in rats, according to the test measuring hypothermic response to 8-OH-DPAT (9).

There are many data indicating the functional antagonism between catecholamine and serotonin systems. Electrophysiological and biochemical studies have revealed an inhibitory role of 5-HT on the function of locus coeruleus noradrenergic neurones (36). Lesions to the raphe nuclei were shown to produce an increase in forebrain concentration of the noradrenaline main metabolite, MHPG. Also it has been reported
that pretreatment with p-chlorophenylalanine (a serotonin synthesis inhibitor) or serotonin receptor blockers, strongly potentiated the excitement after administration of catecholamine-releasing agent – amphetamine (reviewed by Gerson and Baldessarini, (37). Inhibition of serotonergic activity of the median raphe neurones usually results in behavioral and biochemical symptoms clearly resembling effects of microinjections of noradrenaline agonists (38).

Therefore the increased responsiveness of $\alpha_1$-adrenergic receptors following repeated administration of MIR observed in the present study, is likely to result from the overall reduction in the serotonergic (especially 5-HT$_2$) neurotransmission induced by this drug.

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