ANTIDEPRESSANT EFFECT AFTER ACUTE AND SUBACUTE ADMINISTRATION OF NOVEL N-SUBSTITUTED BENZAMIDES ON RESERPINE-INDUCED DEPRESSION IN MICE

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Abstract

The new N-substituted benzamides are structurally related to tiapride, sulpiride or amisulpride, drugs with dual dose-dependent activity on dopaminergic receptors. At low therapeutical doses, these drugs act presynaptically by blocking D3 receptors, which leads to an increase of dopamine level in the synaptic cleft. This mechanism provides utility in the treatment of negative symptoms of schizophrenia and several forms of depression. At higher therapeutical doses, benzamides block postsynaptic D2 receptors, having antipsychotic activity on positive symptoms of schizophrenia.

In this paper we investigated the antidepressant activity of the new synthesized compounds after per os (p.o.) acute and subacute administration in mice with reserpine-induced depression. Pharmacological tests were the tail suspension test (TST) and the antagonism of reserpine-induced blepharoptosis.

In tail suspension test, one of the three investigated compounds had a significant antidepressant effect (P<0.05, ANOVA) after one administration. After eight administrations, two of three new benzamides showed a significant antidepressant effect (P<0.05, ANOVA). The same two compounds produced antagonism toward reserpine-induced ptosis in the mouse, the protection degree being 65.38%, respectively 88.46%.

Rezumat

Noile benzamid e N-substitute sunt asemănătoare structural cu tiaprid, sulpirid sau amisulprid, medicamente care acționează dual, doză dependent, asupra receptorilor dopaminergici. La doze terapeutice mici, aceste benzamide acționează presinaptic, blocând receptorii dopaminergici D3, ceea ce duce la o creștere a nivelului de dopamină în fanta sinaptică, cu utilizare în tratarea simptomelor negative ale schizofreniei sau a unor forme de depresie. La doze terapeutice mari, acțiunea se exercită în teritoriul postsinaptic, cu blocarea receptorilor dopaminergici D2, ceea ce determină activitatea antipsihotică asupra simptomelor pozitive ale schizofreniei.

În lucrarea de față am cercetat acțiunea antidepresivă a trei dintre noii compuși sintetizați, după administrare orală, acută și subacută, utilizând testul suspendării de coadă, la șoarece cu depresie indusă prin rezerpină. De asemenea, a
fost evaluată capacitatea celor trei compuși de a antagoniza ptoza palpebrală indușă prin rezerpină la soarece.

În cazul testului suspendării de coadă, s-a observat efect antidepressiv semnificativ statistic față de lotul martor, pentru unul dintre cei trei compuși investigați, după o singură administrare. După opt administrări zilnice, doi dintre compuși prezintă efect antidepressiv semnificativ statistic comparativ cu lotul martor.

În ceea ce privește testul ptozei palpebrale induse prin rezerpină, doi compuși prezintă un grad de protecție de 65,38%, respectiv 88,46%, ambele rezultate fiind semnificative statistic. Semnificația statistică (P<0.05) a fost determinată prin testele “t” Student și ANOVA.

**Keywords:** benzamides-N-substituted; antidepressant; tail suspension test; reserpine-induced depression; reserpine-induced blepharoptosis.

**Introduction**

The benzamide derivatives amisulpride [2, 11, 12, 14] and sulpiride (5, 10) possess dual dose-dependent activity on dopaminergic receptors. At low therapeutical doses, these drugs act presynaptically by blocking D3 receptors, which leads to an increase of dopamine level in the synaptic cleft. This mechanism provides utility in the treatment of negative symptoms of schizophrenia and several forms of depression. At higher therapeutical doses, benzamides block postsynaptic D2 receptors, having antipsychotic activity on positive symptoms of schizophrenia. Tiapride also was found to be effective in depressive disorders [17]. Thus, substituted benzamides represent an atypical class of antipsychotics, successfully used for both depressive states and schizophrenia.

In our recent research studies [3, 4], we determined a significant antidepressant effect (p<0.05, “t” Student test) for two of the three new synthesized N-substituted benzamides, tested in forced swimming test, after acute administration. LD50 and subacute toxicity were also evaluated.

In this paper we investigated the antidepressant activity of the new synthesized compounds, conventionally named I-5C, I-14C and II-5C [8, 9], after per os (p.o.) acute and subacute administration in mice with reserpine-induced depression. Pharmacological tests were the tail suspension test (TST) and the antagonism of reserpine-induced blepharoptosis.

The structural similarity between tiapride and the new synthesized compounds is shown in Fig. 1:
Figure 1
Chemical structures of tiapride and new N-substituted benzamides

Materials and methods

Male NMRI mice weighing around 20-25 g were supplied by the University of Medicine and Pharmacy “Carol Davila” rodent farm. Groups of 10 animals were housed in plexiglass cages with sawdust litter, having free access to water. The daily food programme was established between 3:00 p.m. and 8:00 a.m., in order to administer all substances on an empty stomach. Experiments were carried out between 9:00 a.m. and 3:00 p.m., the animals being brought in the testing room one hour before the behavioral experiment. The temperature and relative humidity were continuously monitored using an electronic hygro-thermometer. The temperature was between 20ºC and 22ºC and the relative humidity was generally maintained at 35-45%. All procedures were carried out in accordance with the Directive 86/609/EEC of 24th November 1986, regarding the protection of animals used for experimental and other scientific purposes.

Reserpine-induced depression in mice-model

In order to investigate the antidepressant potential of the new synthesized N-substituted-benzamides it was used an animal model for induced depression [6, 7, 15]. The depression model used, evidenced by an increase of the immobility time in the tail suspension test (TST), was obtained after 21 days of per os reserpine administration, in a dose of 1 mg/kg b.w./day [7].
Tail suspension test (TST)

This test is an alternative to the forced swimming test (FST), in which the immobility is induced by suspending the mouse by its tail [16]. Mice were suspended on the edge of a table 50 cm above the floor by the adhesive tape placed approximately 1 cm from the tip of the tail. Being placed in this stressful situation, after initial escape-trying movements, the suspended mouse develops an immobile posture alternating with agitation periods. Antidepressant-like substances are expected to reduce the immobility time in favour to escape-related behavior. The duration of test is 6 min. and the immobility time is manually recorded with a chronometer. All tests were performed 60 minutes after the daily administration.

Antagonism of reserpine-induced blepharoptosis

We used the method originally described by Bourin et al. [1], with modifications. We investigated the effects of 18 daily doses of the three new compounds on reserpine-induced blepharoptosis in mice. In the 18th day, the tested compounds were p.o. administered, 60 min. before the intra peritoneal (i.p.) injection of 1 mg/kg b.w. reserpine. Ptosis rate was evaluated 120 min. after reserpine treatment. The degree of ptosis was rated according to the following rating scale: 0, eyes open; 1, eyes one-quarter closed; 2, eyes half closed; 3, eyes three-quarters closed; 4, eyes completely closed [13]. All results were compared with the scores obtained by the reserpine control group.

Protocol

108 mice were initially tested in TST (t1 moment).

The first part of the experiment consisted of inducing depression in mice using reserpine. For that reason, 90 mice (future depressed groups) received reserpine 1mg/kg b.w./day p.o. and the other 18 mice (blank control group) received distilled water, 0.1 ml/10 g body weight, p.o. The administrations were performed for 21 consecutive days. After this period, all the animals were tested again in TST (t2 moment).

The second part of the experiment consisted of testing the antidepressant effect. The depressed mice were divided into other five groups, taking into consideration the increase of the immobility time after reserpine administration. The purpose was to have about the same average of the immobility time between the groups.

The six new animal groups received daily:

Group 1 – blank control group - distilled water, 0.1 ml/10 g b.w., p.o.;
Group 2 – reserpine control group - distilled water, 0.1 ml/10 g b.w., p.o.;
Group 3 – reference treated group - sulpiride 5 mg/kg b.w. p.o.;
Group 4 – tested treated group – I-5C 42 mg/kg b.w. p.o.;
Group 5 – tested treated group - I-14C 30 mg/kg b.w. p.o.;
Group 6 – tested treated group - II-5C 34 mg/kg b.w. p.o.

The doses for the investigated compounds were established knowing the LD50 values after p.o. administration. We considered that a dose of 1/20 LD50 was appropriate for 21 consecutive daily administrations.

After 1, 8 and 18 days of treatment, all the animals were tested in TST. All tests were performed 60 minutes after the daily administration.

The third part of the experiment consisted of the evaluation of the compounds capacity to antagonise the reserpine induced ptosis. In the 18th day of treatment it has been investigated the compounds ability to antagonise the reserpine induced ptosis. The tested compounds were p.o. administered, 60 min. before the i.p. injection of 1 mg/kg b.w. reserpine. Ptosis rate was evaluated 120 min. after reserpine treatment.

Statistical analysis

Multiple group comparisons were performed using “t” Student test and one-way ANOVA followed by Dunnett’s test, using GraphPad Prism software. When the results distribution wasn’t gaussian, we used the non-parametric Kruskal-Wallis test, followed by Dunn’s test. All results were considered statistically significant when p<0.05.

Results and discussion

Reserpine-induced depression

Table I shows the effect produced by 21 days of reserpine 1 mg/kg b.w. p.o. administration to mice. The immobility time in TST was increased by 86.38% (p<0.0001, unpaired “t” test) compared to the control group.

<table>
<thead>
<tr>
<th>Table I</th>
<th>Effect of 21 days of reserpine treatment on immobility time in the tail suspension test (TST)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Blank control group</td>
</tr>
<tr>
<td>Mean (sec.)</td>
<td>119.67</td>
</tr>
<tr>
<td>SEM*</td>
<td>7.345</td>
</tr>
<tr>
<td>SD**</td>
<td>31.162</td>
</tr>
<tr>
<td>Normal distribution</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Unpaired „t” Student test

| p | <0.0001 |

* SEM – standard error of the mean
** SD – standard deviation
Tail suspension test (TST)

In TST, II 5C was the most active compound, reducing the immobility time with 25.19% after one day administration and with 20.00% after eight consecutive daily doses. Compound I 5C decreased the immobility time statistically significant after eight doses, the effect being 18.52% compared to the reserpine control group. All results are shown in tables II and III.

### Table II

<table>
<thead>
<tr>
<th>Reserpine control group</th>
<th>Sulpiride 5 mg/kg b.w. p.o.</th>
<th>I 5C 42 mg/kg b.w. p.o.</th>
<th>I 14C 30 mg/kg b.w. p.o.</th>
<th>II 5C 34 mg/kg b.w. p.o.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (sec.)</td>
<td>242.17</td>
<td>205.70</td>
<td>196.33</td>
<td>218.00</td>
</tr>
<tr>
<td>SEM</td>
<td>12.101</td>
<td>12.577</td>
<td>18.968</td>
<td>13.793</td>
</tr>
<tr>
<td>SD</td>
<td>33.871</td>
<td>36.190</td>
<td>52.737</td>
<td>55.170</td>
</tr>
<tr>
<td>Normal distribution</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

One way ANOVA

| P                        | < 0.05                      |

Dunnett’s Multiple Comparisons Test

| P                        | NS                          |

| NS                       | NS                          |

| NS                       | < 0.05                      |

Note: NS = non significant

### Table III

<table>
<thead>
<tr>
<th>Reserpine control group</th>
<th>Sulpiride 5 mg/kg b.w. p.o.</th>
<th>I 5C 42 mg/kg b.w. p.o.</th>
<th>I 14C 30 mg/kg b.w. p.o.</th>
<th>II 5C 34 mg/kg b.w. p.o.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (sec.)</td>
<td>200.75</td>
<td>196.94</td>
<td>163.57</td>
<td>205.81</td>
</tr>
<tr>
<td>SD</td>
<td>44.847</td>
<td>33.754</td>
<td>40.899</td>
<td>34.530</td>
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<tr>
<td>Normal distribution</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

One way ANOVA

| P                        | < 0.01                      |

Dunnett’s Multiple Comparisons Test

| P                        | NS                          |

| NS                       | < 0.05                      |

| NS                       | NS                          |

| NS                       | NS                          |

Note: NS = non significant
At 18 days after discontinuation of reserpine administration, the immobility time of the reserpine control group significantly decreased, sign of brain monoamine (noradrenaline and dopamine) reloading. At that moment, the tested compounds showed no significant antidepressant effect compared to the control group and in consequence the experiment was ended.

*Antagonism of reserpine-induced blepharoptosis*

The effect of the new synthesized benzamides on reserpine-induced blepharoptosis is shown in table IV. The most active compounds in this test were I 5C and II 5C, which significantly antagonized the reserpine action.
The effect was 65.38%, respectively 88.46% compared to the reserpine control group. Sulpiride antagonized also the effect of reserpine, the ptosis antagonism effect being 84.62%.

### Table IV
Effect of the new benzamides on reserpine-induced ptosis in mice.
Results are given as mean of the obtained scores

<table>
<thead>
<tr>
<th></th>
<th>Reserpine control group</th>
<th>Sulpiride 5 mg/kg b.w. p.o.</th>
<th>15C 42 mg/kg b.w. p.o.</th>
<th>114C 30 mg/kg b.w. p.o.</th>
<th>II 5C 34 mg/kg b.w. p.o.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (score)</td>
<td>2.6</td>
<td>0.4</td>
<td>0.9</td>
<td>2.0</td>
<td>0.3</td>
</tr>
<tr>
<td>SEM</td>
<td>0.163</td>
<td>0.221</td>
<td>0.179</td>
<td>0.258</td>
<td>0.213</td>
</tr>
<tr>
<td>SD</td>
<td>0.516</td>
<td>0.699</td>
<td>0.567</td>
<td>0.816</td>
<td>0.675</td>
</tr>
<tr>
<td>Normal distribution</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Kruskal-Wallis Test</td>
<td>&lt; 0.0001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dunn’s Multiple</td>
<td>&lt; 0.001</td>
<td>&lt; 0.05</td>
<td>NS</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Comparisons Test</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: NS = non significant

The ptosis observed in reserpine control group is a consequence of depletion of monoamines (noradrenaline, serotonin or dopamine). The significant antagonistic effects produced by sulpiride and two of the tested benzamides suggest that these compounds can increase the brain monoamine level.

### Conclusions
It has been evaluated the antidepressant effect of three new benzamides after acute and subacute administration in depressive mice. Depression was induced by 21 days of 1 mg/kg b.w. p.o. reserpine administration and was assessed by the increase of the immobility time compared to saline group in TST (+86.38%; p<0.0001).

The most active compound was II 5C, which produced a significant decrease of the immobility time with 25.19% after one day administration and with 20.00% after eight consecutive daily doses. Compound I 5C reduced the immobility time statistically significant after 8 doses, the effect being 18.52% compared to the reserpine control group. Benzamides I 5C and II 5C significantly antagonized the reserpine-induced blepharoptosis, the effect being 65.38%, respectively 88.46% compared to the reserpine control group.

In conclusion, our results suggest that two of the new synthesized benzamides produced antidepressant-like effect in mice in TST and blepharoptosis test. The mechanism we proposed is similar with sulpiride.
administered in low doses, which acts on presynaptic dopaminergic D₃ autoreceptors, but this assumption needs further evaluation.

References

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