The antidepressant effect of an antiulcer pentadecapeptide BPC 157 in Porsolt’s test and chronic unpredictable stress in rats. A comparison with antidepressants


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Abstract — Various antidepressants have antidepressant activity. Likewise, the models currently used in ulcers and depression disorders research have a considerable degree of similarity. Therefore, the possibility that depression disorders could be effectively influenced by a primary antiulcer agent with a cyto/organoprotective activity, such as the novel stomach pentadecapeptide BPC 157, was investigated in two rat depression assays. First, a forced swimming test (a Porsolt’s procedure) was used. As a more severe procedure, chronic unpredictable stress (after 5 d of unpredictable stress protocol, once daily drug application during stress procedure, open field-immobility test assessment at fourth or sixth day of medication) was used. In a forced swimming test, a reduction of the immobility time in BPC 157 (10 μg, 10 ng·kg¹ i.p.) treated rats corresponds to the activity of the 15 mg or 40 mg (i.p.) of conventional antidepressants, imipramine or nialamide, respectively, given according to the original Porsolt’s protocol. In chronic unpredictable stress procedure, particular aggravation of experimental conditions markedly affected the conventional antidepressant activity, whereas BPC 157 effectiveness was continuously present. The effect of daily imipramine (30 mg) medication could be seen only after a more prolonged period, but not after a shorter period (i.e., 4-d protocol). In these conditions, no delay in the effectiveness was noted in BPC 157 medication and a reduction of the immobility of chronically stressed rats was noted after both 4 and 6 d of BPC 157 (10 μg, 10 ng) medication. © 2000 Elsevier Science Ltd. Published by Éditions scientifiques et médicales Elsevier SAS

1. Introduction

One of the possible intriguing relationships may be the connection between ulcer disease and behaviour disturbances such as depression [10, 11]. As in depression, antidepressants are effective in relieving gastrointestinal lesions, both experimentally and clinically [5, 9, 12, 16, 31]. On the other hand, the opposite possibility that agents primarily designated as antiulcer drugs could vice versa be also effective in depression disorders has not been so far investigated. Based on its salutary effect on gastrointestinal lesions induced by various challengers [4, 13, 17-30, 33-36], the novel stomach (antiulcer) pentadecapeptide, coded BPC 157 could be a valuable candidate. Besides, the possible role of such neuropeptides as somatostatin, neuropeptide Y, and corticotropin, which are closely associated with the biogenic amines transmission, is already postulated in both conditions [8, 12]. Very recently, it was shown that BPC 157, which has no influence on gross behaviour of normal animals, blocks the stereotypy produced acutely by amphetamine in rats, and the development of haloperidol-induced supersensitivity to amphetamine in mice [6]. Thus, it seems likely that pentadecapeptide BPC 157 is also associated with the biogenic amines transmission [6]. In addition, the evidence that this pentadecapeptide would particularly interact with the catecholaminergic system, and particularly the central dopamine system, had been already noticed in this pentadecapeptide protection of gastric mucosa during stress [27]. A significant pool of BPC has been immunohistochemically shown in the stomach and also in the brain [21].

Therefore, the focus was on the mentioned pentadecapeptide, BPC 157, given in its known antiulcer regimen [21, 23, 25-30], and its efficacy on animal depression was evaluated in different experimental models [37-39] in comparison with conventional agents. Finally, considering the likely significance of the serotonin system for depression disorders, and the not fully elucidated importance of most serotonin in gastrointestinal tract (i.e., 90% of total serotonin) [32], the possibility that the stomach pentadecapeptide BPC 157 could have a beneficial influence in depression models may not be entirely unexpected.

2. Materials and methods

2.1. BPC 157. Preparation of the peptide

The pentadecapeptide BPC 157 (Gly Glu Pro Pro Pro Gly Lys Pro Ala Asp Asp Ala Gly Leu Val), M.W.
1 419 freely soluble in water at pH 7.0 and in saline, was prepared as described in detail previously [4, 6, 19–30]. A peptide with 99% (HPLC) purity (1-des-Gly peptide as impurity) was used.

2.2. Animals

Female, Albino rats of Fisher-344 strain, 180–200 g b.w. were used in all of the experiments. They were housed in single cages 5 d before the beginning of the experiments. Food and water were available, 12 h light/dark cycles were used. Twelve to fourteen rats were used per experimental groups.

2.3. Procedure and drugs application

The rats were individually forced to swim inside a vertical glass cylinder (as described by Porsolt [14, 15] (for review see e.g., [38])) (height: 40 cm; diameter: 18 cm) containing 20 cm of water maintained at 24–25 °C. After 15 min (pre-test session), the rats were dried for 15 min. They were individually re-placed in the cylinder 24 h later (same conditions) and the total duration of immobility (passive floating, slightly hunched, upright position, the head just above the surface) was measured during a 5-min test, by a well trained observer, unaware of the treatment. In randomly assigned rats, the agents (per kg b.w. i.p.), given in their usual regimens [6, 25–28, 38], BPC 157 (10 μg, 10 ng), nialamide (40 or 80 mg) (Pliva, Zagreb, Croatia), imipramine (15 or 30 mg) (Pliva, Zagreb, Croatia), were applied three times at 24, 3 h and 1 h before the 5-min test session, whereas controls simultaneously received an equivolume (5 mL kg⁻¹) of 0.9% NaCl.

In chronic unpredictable stress assay (for review, see e.g., [38]), the rats were immersed for 90 min in water at 24–26 °C for five consecutive days at different times (day 1: 9.00 a.m.; day 2: 11.00 a.m.; day 3: 4.00 p.m.; day 4: 7.00 a.m.; day 5: 1 p.m.). One hour after the immersion 9.45 a.m.–11.15 p.m., noise for 9 h (6.00 p.m.–3.00 a.m.); 11th day: dark 6.00 a.m.–6.00 p.m., light 6.00 p.m.–6.00 a.m., noise for 30 min (7.00–7.30 p.m.), water immersion 6.00 p.m.–7.30 p.m. The medication (per kg b.w.) (BPC 157 10 μg, 10 ng, imipramine 30 mg or saline) was daily applied i.p. regularly at 1.00 p.m. One hour after the immersion procedure at the fourth and sixth day of therapy (9th and 11th day of chronic stress procedure), all the rats were again individually studied on the open field-immobility test, and the number of passed squares was assessed as described before. Naive, healthy animals, treated with saline only, served as an additional control group.

2.4. Statistical analysis

Dunnett’s (two tailed test) (Porsolt’s procedure), Kruskal-Wallis and Mann-Whitney U-test (chronic stress procedure) were used for statistical comparison. P-values of 0.05 or less were considered to be statistically relevant.

3. Results

3.1. Porsolt’s procedure

The applied procedure produced a significant period of immobility in all of the control saline rats (table I). Imipramine and nialamide, given in their usual regimens, used as reference agents, significantly reduced the otherwise consistently raised immobility time in rats subjected to Porsolt’s procedure, along with the previously described data (i.e., [38]). An essentially similar effect was noted with pentadecapeptide BPC 157 application (i.e., BPC 157 10 μg and 10 ng kg⁻¹ b.w. vs. imipramine 15 and 30 mg or nialamide 40 and 80 mg kg⁻¹ b.w.).

3.2. Chronic unpredictable stress

A prolonged stress procedure using various unpredictable and inescapable challenges produced a strong increase of the immobility compared with normal rats in practically all stressed rats. Thus, these rats, having less than 40% mobility of healthy rats, were randomly assigned for further treatment, whereas the others were excluded from further experiment. The raised immobility was found sustained in chronically stressed
controls, treated with saline only (a tendency towards further deterioration along with continuation of stress procedure seems to be present) (table II).

In comparison with the Porsolt’s procedure, this chronic stress-immobility may be more resistant to the applied medication (given daily in the regimen shown to be effective in Porsolt’s procedure), assessed either at the fourth or sixth day of medication. Chronic stress-immobility was not easily antagonized by imipramine, since it could not improve animal behaviour before the sixth day of medication, administered daily in its higher dose (i.e., 30 mg·kg⁻¹ b.w. i.p.). In contrast, BPC 157 was effective, and its salutary effect was only dose-dependently reduced, but consistently evident at any of the tested intervals. Assessed after either 4 or 6 d of BPC 157 medication, a clear reversal of otherwise sustained immobility in chronically stressed rats towards the values regularly observed in naive, non-stressed rats, was clearly noted. Interestingly, the rats challenged with the BPC 157 ng-regimen appeared to be also significantly more mobile when compared with the controls. However, BPC 157 ng-dosage became less effective than the BPC 157 µg-regimen (in Porsolt’s procedure both dosages produced a similar improvement).

4. Discussion

In these experiments, pentadecapeptide BPC 157, primarily designated as an antiulcer agent with cyto/organoprotective activity [21, 23, 25-30], was shown to reduce the duration of immobility in a way that might be typical of the re-uptake (serotonin, noradrenaline) of inhibitor antidepressants [38]. Of note, a positive effect was consistently observed in both of the tested models. In general, these findings were obtained in these two distinctive models, using the same doses of tested agents, pentadecapeptide BPC 157, and reference agents. Consequently, it seems likely that these salutary effects are not accidental. This may suggest a particular action, specially in the light of the previously mentioned recently described pentadecapeptide BPC 157 effects on variously disturbed animal behaviour [6].

Considering these intriguing findings, an argument about the possible profile of the pentadecapeptide BPC 157 antidepressant activity has to be considered. As noted in the lower dosage range compared to conventional antidepressants (µg-ng vs. mg in Porsolt’s procedure), an improvement in antidepressant activity could probably be speculated. By contrast, a false positive result could be possible too. Namely, besides antidepressants, various agents, such as amphetamine-like agents, may reduce the duration of immobility in the forced swimming test. However, this may be highly unlikely. Considering the noted efficacy of the tested pentadecapeptide, psychostimulants efficacy

### Table I. Porsolt’s forced swimming test and the effect of pentadecapeptide BPC 157 and antidepressants. The agents were applied i.p. three times at 24 h, 3 h and 1 h before the 5 min-test session, whereas controls simultaneously received an equivolume (5 ml/kg) of the 0.9 % NaCl.

<table>
<thead>
<tr>
<th>Dose per kg b.w., i.p.</th>
<th>Immobility time (sec)</th>
<th>Means ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>155.5 ± 7.2</td>
<td></td>
</tr>
<tr>
<td>Imipramine 15 mg</td>
<td>115.1 ± 5.5*</td>
<td></td>
</tr>
<tr>
<td>Imipramine 30 mg</td>
<td>110.4 ± 10.7*</td>
<td></td>
</tr>
<tr>
<td>Nialamide 30 mg</td>
<td>113.4 ± 5.8*</td>
<td></td>
</tr>
<tr>
<td>Nialamide 40 mg</td>
<td>103.9 ± 11.3*</td>
<td></td>
</tr>
<tr>
<td>BPC 157 10 µg</td>
<td>80.9 ± 7.4*</td>
<td></td>
</tr>
<tr>
<td>BPC 157 10 ng</td>
<td>78.1 ± 7.5*</td>
<td></td>
</tr>
</tbody>
</table>

* P < 0.05, at least, vs. control (saline 5 ml/kg i.p.).

### Table II. Chronic unpredictable stress. Number of squares entered. The effect of pentadecapeptide BPC 157 and imipramine when given once daily in chronically stressed rats with established immobility.

<table>
<thead>
<tr>
<th>Therapy (kg b.w. i.p.)</th>
<th>Measure</th>
<th>Days after beginning of medication</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>4-d medication</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Number of crossings</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>Min</td>
</tr>
<tr>
<td>Imipramine 30 mg</td>
<td>U = 51</td>
<td>12</td>
</tr>
<tr>
<td>BPC 157 10 µg*</td>
<td>U = 0.0</td>
<td>42</td>
</tr>
<tr>
<td>BPC 157 10 ng*</td>
<td>U = 3.0</td>
<td>18</td>
</tr>
</tbody>
</table>

+ vs. control (saline 5 ml/kg i.p.) sixth day; * vs. control (saline 5 ml/kg i.p.) fourth and sixth day.
was noted in markedly higher doses (i.e., mg vs. µg-ng) [38]. Furthermore, as mentioned before, this pentadecapeptide, particularly interacting with the central dopamine system (i.e., in protection of gastric mucosa during stress) [27], also blocks the stereotypy produced acutely by amphetamine in rats. Likewise, this pentadecapeptide inhibits the development of haloperidol-induced supersensitivity to amphetamine in mice. Together, these data were interpreted as a particular modulatory activity of this pentadecapeptide rather than an interaction at receptor sites [6]. Theoretically, such an activity may be favourably used for the antidepressant activity. Moreover, unlike psycho-stimulants, pentadecapeptide BPC 157 does not produce enhanced activity, nor alteration in normal body temperature in rodents [6, 20]. For the control condition additional satisfaction, the animals were injected with the pentadecapeptide according to the same treatment schedule used in the above described experiments. No locomotor hyperactivity is produced under the same test condition (data not shown). Finally, in support of a specific, probably antidepressant activity of the tested pentadecapeptide, these data have to be viewed considering the suggested special positive relationship of the forced swimming test with the clinical potency of antidepressants (r = 0.58, P < 0.05, calculated using Porsolt’s data) [38].

Likewise, these complaints seem to be at least partly solved by using the chronic unpredictable stress procedure as an additional meaningful assay [7, 38]. In this model, a close correspondence to central symptoms of endogenomorphic depression has been emphasized [38]. A lack of reactivity to an acute stress along with a failure to respond to (presumably) pleasure stimuli have been particularly recognized as specific advantages favouring such a similarity [38]. As an important point in our study, the treatment was started after chronic stress induction (i.e., in the majority of studies, a prophylactic treatment was involved [38, 39]), and carried out during the sustained severe stress conditions. Interestingly, imipramine could reverse the immobility in chronically stressed rats when applied for 6 but not for 4 d only. Pentadecapeptide BPC 157 could reduce immobility in both an earlier and later interval, as could be seen after 4 or 6 d of medication. Considering the existence of BPC 157, it has unusual stability [21]: incubated in human gastric juice or in water, unlike standard peptides, this pentadecapeptide was not subjected to any degradation at least for 24 h [35]. This could explain its prolonged activity in chronic stress experiments.

Therefore, seen from a methodological point of view, together with Porsolt’s procedure, these results would provide a promising basis. Could a likely stronger antidepressant activity of this pentadecapeptide be indeed suggested? In chronic stress procedure, a threatening shift towards more rigorous and more sustained negative conditions (i.e., eleven subsequent days of chronic stress vs. 24 h in Porsolt’s test) could be clearly pointed out. An initiation of the medication in apparently more vigorous experimental conditions which provoke animal disturbance (i.e., prolonged subsequent water immersions, besides other unpredictable stresses (h), vs. short swimming intervals (min)) should be emphasized. Apparently, in chronic stress procedure, the agents were given once daily contrasting with three time applications during 24 h of the Porsolt’s procedure. Together, these circumstances may per se challenge beneficial capability of the tested agents. The imipramine positive effect noted just in the later interval (and not the earlier one) could be theoretically due to the generally known postponed efficacy of the antidepressants [38, 39]. In these conditions, no similar delay could be seen in the pentadecapeptide effectiveness. This pentadecapeptide beneficial activity would only be dose-dependently reduced (the lower dose of the 10 ng·kg⁻¹ became less effective than the 10 µg·kg⁻¹ dose) and unlike imipramine, it was present at any of the tested intervals. When noted, this salutary effect of the tested pentadecapeptide appears to be quite persistent: the last medication had been applied many hours before the immobility was tested in the open, whereas a stable reversal of the otherwise sustained immobility in chronically stressed animals was seen after either 4 or 6 d of the pentadecapeptide medication. Thus, taken together these findings, an effect, at least partly, different from the antidepressants catecholamine re-uptake inhibition may be possible. Besides, an acute toxicology shows a very high therapeutic index of the pentadecapeptide BPC 157, since also very high dosages were not accompanied by death or pathologic changes. Interestingly, unlike antidepressants cardiotoxicity (i.e., [3]), this pentadecapeptide has a protective effect found in hypoxic and reoxygenation injury in the isolated guinea pig heart [1], and it prevents antidepressants-arrhythmia (i.e., [3]) in rats [21]. For instance, BPC 157 clearly prevents sudden decrease in heart rate and conduction disturbances induced by desipramine (10 mg·kg⁻¹) i.e., PQ prolongation and QRS widening, ventricular tachycardia associated with proarrhythmic effect of desipramine, and severe atrioventricular block [21].

Whether this salutary effect of the pentadecapeptide BPC 157 appeared consequently of the possible relationship between these two disorders or not remains to be further determined. The point was thus made that an antiulcer drug, such as pentadecapeptide BPC 157, could act by opposing in some way, an unidentified process, that manifested itself in different ways in different sites. Although this hypothesis may be coun-
terproductive, besides the mentioned clinical evidence [31], many indications accord with such a suggestion.

In accordance with common experimental ulcer/depression terms [10-12], the aggravation of stress conditions and/or prolongation of the stress period would likely pick out the most effective agents in ulcer research. Seen from a general point of view, the essentially similar experimental procedures known to induce either gastrointestinal ulcer (for review see e.g., [12]) or depression (e.g., [38]) apparently point to a considerable degree of parallelism. Along with a convincing characterization of individual animals or particular rat strains (e.g., Wistar Kyoto), as both more depressed and ulcer prone, it is known that a prior stress/shock experience, particularly unpredictable and inescapable – as in the present report – regularly aggravates both gastrointestinal lesions and depression patterns (e.g., learned helplessness, freezing behaviour) in animals [10, 11].

In this, if both the gastrointestinal lesions and the depression disturbances are taken as stress disorders, the noted positive effect of the stomach pentadecapeptide BPC 157 in the same dosage range in animal depression as in gastrointestinal lesion assay [21, 23, 25-29] could be favourable at least from a theoretical viewpoint. As mentioned, in line with this and the importance of the serotonin system for depression disorders, it should be considered that more than 90% of the endogenous serotonin is found in the gastrointestinal tract, with the highest concentration in the stomach [32]. Although the full pathophysiological importance of this serotonin concentration is still obscure [32] and the significance of pentadecapeptide BPC 157 remains to be further determined, it could be speculated that a particular conjunction and modulation of these systems could be at least partly responsible for the observed BPC 157 beneficial effects. On the other hand, immobilization stress, a long-held ulcerogenic procedure, could induce a decreased brain-serotonin synthesis as well [2].

Thus, seen from this point of view, it could hardly be that this salutary activity is an exception between antiulcer agents. Admittedly, all these indications could be likely afforded if an antidepressant activity may be proved for other antiulcer agents.

References


