

## Pentadecapeptide BPC 157 attenuates chronic amphetamine-induced behavior disturbances

Predrag SIKIRIC<sup>1</sup>, Nikola JELOVAC, Andjelka JELOVAC-GJELDUM, Goran DODIG, Mario STAREŠINIC, Tomislav ANIC, Ivan ZORICIC, Davor RAK, Darko PEROVIC, Gorana ARALICA, Gojko BULJAT, Ingrid PRKACIN, Martina LOVRIC-BENCIC, Jadranka SEPAROVIC, Sven SEIWERTH, Rudolf RUCMAN, Marijan PETEK, Branko TURKOVIC, Tihomil ZIGER, Alenka BOBAN-BLAGAIC, Vlado BEDEKOVIC, Ante TONKJIC, Slaven BABIC

(Department of Pharmacology, Medical Faculty University of Zagreb, Croatia)

**KEY WORDS** BPC 157; amphetamine; stereotyped behavior

### ABSTRACT

**AIM:** To investigate the effect of pentadecapeptide BPC 157 on chronic exposure to amphetamine in rats, particularly the changes commonly referred in chronic amphetamine studies as tolerance (lesser grade of stereotyped behavior, without increased excitability) and reverse tolerance (ie, prominent stereotyped behavior and heightened startle response upon late amphetamine challenges). **METHODS:** After initial application (initial single dose-regimen), amphetamine (10 mg/kg, ip) was given once daily till d 5 (continuous administration-regimen), and thereafter on d 8, 16, and 46 (intermittent administration regimen). For stereotyped behavior and heightened startle response the observation period was 120 min after amphetamine application, and each animal was observed for 10 s in 5 min intervals. Pentadecapeptide BPC 157 (10 µg/kg or 10 ng/kg, ip) or saline (5.0 mL/kg, ip) were given only at the beginning of the experiment, simultaneously with the initial dose of amphetamine. **RESULTS:** In relation to applied initial-single/continuous/intermittent amphetamine applications regimen, the control amphetamine rats throughout the experiment showed the changes in stereotyped behavior and heightened startle response, increment or decrement, commonly explained in chronic amphetamine studies as tolerance and reverse tolerance.

After the initial application of the amphetamine, the higher BPC 157 dosage apparently attenuated the stereotyped behavior, while the lower dosage of BPC 157 did not reach a statistical significance. Considering the forthcoming amphetamine challenges, in the rats initially treated with pentadecapeptide BPC 157, either 10 µg- or 10 ng-dose, at the time of the first application of amphetamine, the stereotyped behavior remains to be attenuated after all additional amphetamine challenges (on d 2-5, 8, 16, and 46). This attenuation was not limited to stereotyped behavior only. After the initial application of the amphetamine the heightened startle response was also apparently mitigated in rats receiving the BPC 157 dosage, either higher or lower. Later, confronted with the forthcoming amphetamine challenges, they showed apparently less abnormal excitability at all tested points. **CONCLUSION:** In summary, gastric pentadecapeptide BPC 157 (ie, both µg- and ng-BPC 157 regimens) attenuated chronic amphetamine disturbances. This effect was present throughout the observation period at a statistically significant level. Therefore, it seems that this gastric pentadecapeptide BPC 157 has a modulatory effect on dopamine system, and it could be used in chronic amphetamine disturbances.

### INTRODUCTION

It was longly noticed that gut peptides might strongly interact with dopamine system<sup>1</sup>. If injected in rats, amphetamine promptly induces characteristic stereotypes (compulsive sniffing, licking, and gnawing)<sup>2,3</sup> because of the activation of dopaminergic system in the corpus striatum<sup>4</sup>, while continuing amphetamine administration induces complex changes, increment or decrement, commonly referred in chronic amphetamine studies as tolerance and reverse tolerance<sup>5-9</sup>. Recently, it was

<sup>1</sup> Correspondence to Prof Predrag SIKIRIC, MD, Ph D.  
Department of Pharmacology, Medical Faculty University of Zagreb, Salata 11, POB 916, 10000 Zagreb, Croatia.  
E-mail sikiric@mef.hr  
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demonstrated that a gastric pentadecapeptide, GEPPPGK-PADDAGLV,  $M_r$  1419, coded BPC-157<sup>[10]</sup>, evidently attenuated amphetamine induced stereotyped behavior and heightened startle response of rat in acute experiments. Now, it was shown that in the acute experiments, pentadecapeptide BPC 157 also attenuated the harmful effects in chronically amphetamine-treated rats.

The background is that the opposing effect of pentadecapeptide BPC 157 is probably especial, since it blocks also a postponed increased effect of amphetamine following haloperidol administration, amphetamine climbing behavior. This haloperidol/amphetamine effect is commonly accepted as a delayed result of striatal dopamine receptors up-regulation following dopamine antagonist haloperidol application and dopamine receptors blockade<sup>[10]</sup>, that causes an over-effect upon amphetamine challenge. Likewise, pentadecapeptide BPC 157 may also counteract immediate consequences of dopamine receptors blockage, catalepsy, and/or somatosensory disturbance<sup>[11]</sup>, given simultaneously with dopamine receptor blockers haloperidol, fluphenazine, sulpiride, and clozapine. Besides these, it attenuated motoric disturbances induced by neurotoxin 1-methyl-4-phenyl-1, 2, 3, 6-tetra-hydropyridine (MPTP), a Parkin-songenic neurotoxin, affecting nigrostriatal dopamine<sup>[12]</sup>, or reserpine, a depletor of dopaminergic intraneuron granules<sup>[12]</sup>. Finally, with a potential antidepressant activity<sup>[13]</sup>, pentadecapeptide BPC 157 counteracted helpless behavior in rats subjected to Porsolt's forced swimming test and immobility in open field chronically stressed rats. Very recently, given pentadecapeptide BPC 157 simultaneously with diazepam, in studies of diazepam tolerance/withdrawal a lack of tolerance development was demonstrated in tolerance studies, while residual anticonvulsive activity was prolonged, and physical dependence/withdrawal hallmark postponed<sup>[14]</sup>. Noteworthy, GABA-nergic transmission is longly implicated in the regulation of dopamine mediated events within extrapyramidal system and behaviors dependent on striatal functions (catalepsy, stereotypes)<sup>[15, 16]</sup>. Likewise, pentadecapeptide BPC 157 showed a particular anxiolytic activity (ie, light/dark, shock probe/burying test)<sup>[17]</sup>.

Therefore, this gastric pentadecapeptide BPC 157 was a particular tool for investigation of chronic amphetamine administrations in rats. The present study extends previous findings, investigating its effect on the chronic amphetamine applications in rats. Following initial single amphetamine administration, a prolonged

amphetamine protocol included firstly continuous daily administration, and thereafter, after a withdrawal period, intermittent application of amphetamine at the particular later time points<sup>[5-9]</sup>.

## MATERIALS AND METHODS

**Drugs** Pentadecapeptide BPC 157 (GEPPPGK-PADDAGLV,  $M_r$  1419) (manufactured by Diagen, Slovenia) is a partial sequence of human gastric juice peptide BPC freely soluble in water at pH 7.0 and in saline, prepared as described before<sup>[10-11, 17-20]</sup> and dissolved in saline. Peptide with 99 % (HPLC) purity (1-des-Gly peptide as impurity, biologically inactive) was used. Amphetamine (*d*-amphetamine-sulfate, Sigma, USA) was dissolved in saline, whereas haloperidol (Sigma, USA) was dissolved in saline.

**Animals** Male Wistar Albino rats weighing 200 - 250 g, randomly assigned were used in the experiments. Rats were normally housed in groups of 12 with free access to food and water in a temperature and humidity controlled room under a 12 h light/12 h dark cycle.

### Amphetamine stereotypy and excitability

Previously used procedure was carried out<sup>[10]</sup>. Briefly, for observation the rats were placed in individual perspex cages, measuring 30 cm × 20 cm and 15 cm high, in a sound proofed, diffusely illuminated room maintained at a temperature of 20 - 22 °C. Cardboard screens were placed between the cages to prevent rats being influenced by their neighbours. Rats were placed in the observation cages 30 min before drug treatment to allow adaptation to the new environment<sup>[30]</sup>. All observations were made between 10:00 and 19:00 by a trained observer, unaware about the given treatment. The observation period was 120 min after amphetamine application, and each animal was observed for 10 s in 5 min intervals<sup>[3]</sup>.

**Experimental protocol** On d 1, pentadecapeptide BPC 157 (10 µg/kg or 10 ng/kg, ip) or saline (5.0 mL/kg, ip) were given simultaneously with the first application of the amphetamine (10 mg/kg, ip). Thereafter, amphetamine regimen (using the same dose) was continued as follows: till d 5, once daily (conditioning regimen by continuous administration); thereafter, after a withdrawal period, as an intermittent treatment (challenging period) at particular later time points, on d 8, 16, and 46 (Fig 1).

The intensity of stereotypes was assessed by the scale used in our recent report<sup>[10]</sup>, proposed by Costall and

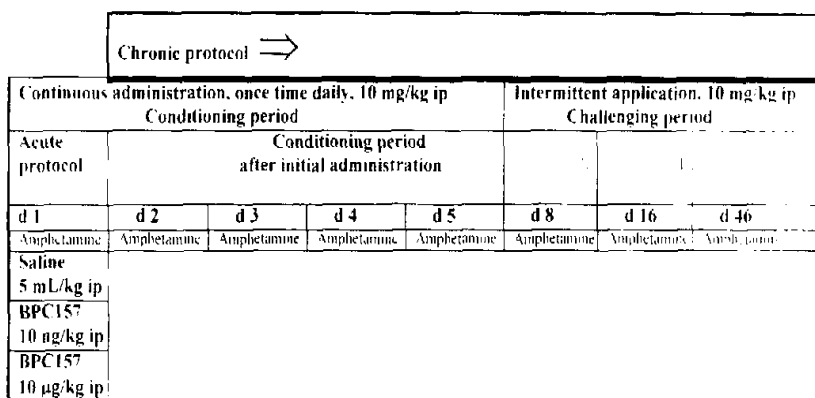


Fig 1. Experimental protocol.

Naylor (1972)<sup>[2]</sup>, and similarly used in other studies<sup>3,29</sup>. Briefly, the effects were assessed using a scoring system (0-4) as follows: 0, the appearance of the animals is the same as saline treated animals; 1, discontinuous sniffing, constant exploratory activity; 2, continuous sniffing and small head movements, periodic exploratory activity; 3, continuous sniffing, discontinuous biting, gnawing or licking, brief periods of locomotor activity; 4, continuous gnawing, biting and licking, no exploratory activity. Besides the intensity of stereotypes, the excitability of animals was assessed as described in our previous study<sup>[10]</sup>, by their reactivity to the uniformed acoustic stimulus of medium intensity. It was scored by a simple scale; 0, no fear reaction; 1, single twitch; 2, stronger twitching, jumping, and escaping; 3, violent twitching, panic jumping, and escaping.

**Statistical analysis** The stereotypy scores obtained after amphetamine administration at each time point for rats cotreated with saline and those cotreated with BPC 157 (both dosages) were compared using Kruskal-Wallis ANOVA followed by Mann-Whitney test. The excitability scores and scores obtained in the climbing assay were compared in the same way. The differences were considered to be significant at  $P < 0.05$  (downward adjustment because of the multiple comparison)<sup>[30]</sup>.

## RESULTS

### General

**Stereotyped behavior** In general, subjected to described initial-single/repeated-intermittent amphetamine application (s)-regimen, amphetamine-controls showed

some common characteristics. Stereotyped behavior was present at the all of the tested intervals. Development was rapid (stereotyped behavior was noted already 5 min following amphetamine application), while deterioration occurred through forthcoming intervals. The only exception was after the final application (ie, on d 46 further aggravation was absent). On the other hand, with respect to other amphetamine parameters after the first initial application, the rats confronted with subsequent amphetamine challenge (s), might react differently.

**Heightened startle response** Like in the case the stereotyped behavior the intensity of abnormal excitability, ie, heightened startle response varied after initial first application. However, the course of the heightened startle response in chronic amphetamine-treated rats seemed to be particular and different from the course noted in stereotyped behavior. Unlike stereotyped behavior, heightened startle response was not always present, and it might be noted only at particular intervals, while the rest was characterized by the behavior indistinguishable for normal behavior. This may suggest that the reaction leading to heightened startle response was more limited in its capacity, and it could not be always elicited like stereotyped behavior.

### Initial application at the beginning of the conditioning period

**Stereotyped behavior** The most exaggerated disturbance, ie, continuous gnawing, biting and licking, no exploratory activity (score 4) was seen between 30 and 110 min. Continuous sniffing, discontinuous biting, gnawing or licking, brief periods of locomotor activity (score 3) were seen at earlier (ie, 20-25 min)

and at later intervals (115 – 120 min). Less disturbed behavior, such as *continuous sniffing and small head movements*, periodic exploratory activity, assessed as a score 2, was seen immediately after amphetamine application (ie, at 5 min-assessment). These findings are consistent with the disturbance level seen after an acute administration<sup>[10]</sup>.

After the initial application of the amphetamine, in the rats receiving the higher BPC 157 dosage (10 µg) the stereotyped behavior was apparently attenuated during 5 – 120 min ( $P < 0.05$ ), while in rats treated with the lower dosage of BPC 157 (10 ng) statistical significance was not found. These differences were present throughout the observation period and were statistically significant at all the observed intervals (Fig 2A).

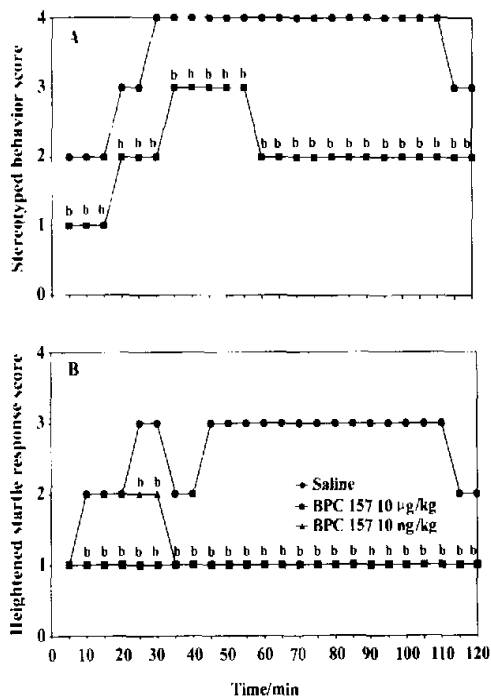


Fig 2. Effect of pentadecapeptide BPC 157 on amphetamine disturbances at the beginning of the conditioning period (d 1). Stereotyped behavior (Fig 2A); heightened startle response (Fig 2B).  $n = 6$ .  $^bP < 0.05$  BPC 157 vs saline.

**Heightened startle response** In naive rats, the heightened startle response assessed by their reactivity to the uniformed acoustic stimulus of medium intensity, was soon seen after amphetamine application in all control rats. The disturbed behavior characterized by a violent twitching, panic jumping and escaping (score 3)

appeared through the experiments at 25 – 30 min. 45 – 110 min intervals, the rest was stronger twitching and jumping (score 2). Like in the stereotyped behavior, this seems to be particularly related to amphetamine application, and it could not be seen in non-amphetamine treated rats. On the other hand, saline-treated naive animals showed always either no fear reaction (score 0), or single twitch (score 1) (data not specifically shown), that may be occasionally noted in amphetamine rats as well, before or after major disturbance. For instance, at the earliest period immediately after amphetamine application, the controls showed single twitch. Thereafter, these disturbances appeared to be quite long-lasting, and increasingly evident after some period (eg. min), and they could be regularly seen till the end of the observation period (120 min).

After the initial application of the amphetamine, in rats receiving the BPC 157 dosage, either 10 µg/kg during 10 – 120 min or 10 ng/kg during 25 – 120 min, the heightened startle response was apparently mitigated ( $P < 0.05$ ). These differences were present throughout the observation period and were statistically significant at all the observed intervals (Fig 2B).

#### Conditioning period after the initial application

**Stereotyped behavior** On d 2, the maximal amphetamine-reaction was shifted toward the earlier intervals (25 – 85 min). At the remaining period the stereotyped behavior assessed as a score 3 was noted, while the behavior assessed as score 2 did not occur. Thus, relative to the initial challenge the amphetamine reaction is more rapid and more prominent. After this time, the reaction toward amphetamine appeared to be gradually lessened, despite continuous applications (Fig 3A). On d 3 and d 4, the reaction was still prominent, the period of maximal disturbance was between 25 and 65 min (d 3, Fig 3B) or 50 – 65 min (d 4), remaining period was assessed as score 3 (Fig 3C). Finally, at the end of the conditioning period, on d 5, the reaction was evidently attenuated; the most disturbed behavior achieved only the score 3 at 35 – 110 min interval. Besides this, the rats showed disturbance assessed as score 2, before (10 – 30 min), and after (115 – 120 min). The highest disturbance, scored with maximal score (score 4), that had been previously seen regularly after amphetamine application, was not observed again. Discontinuous sniffing, constant exploratory activity regarded as the lowest level of stereotyped behavior, calculated as a score 1, was evidenced at the earliest

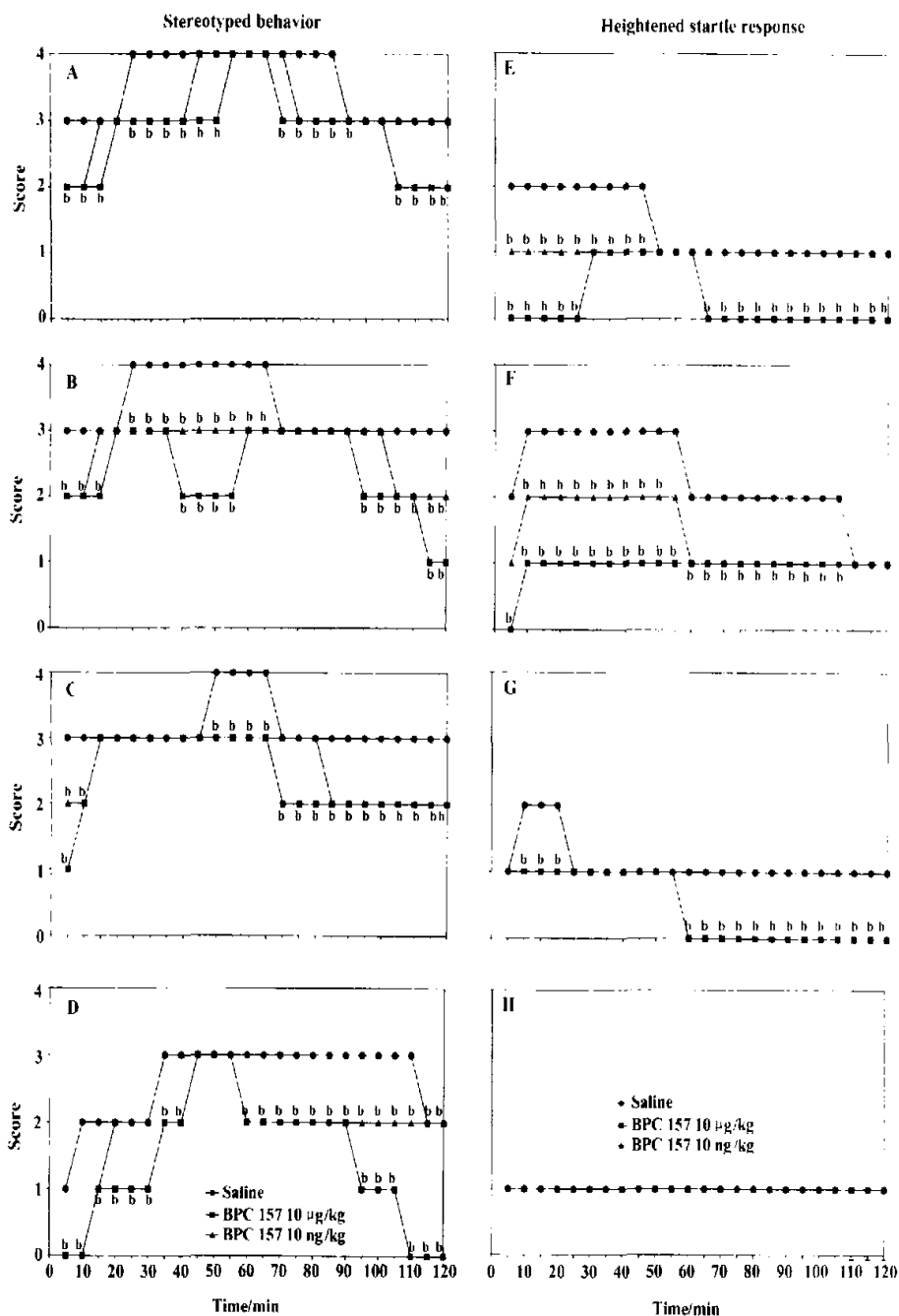


Fig 3. Effect of pentadecapeptide BPC 157 10 µg/kg or 10 ng/kg ip on amphetamine disturbances during conditioning period. A, E: d 1-2; B, F: d 1-3; C, G: d 1-4; D, H: d 1-5. n=6. \*P<0.05 vs saline.

period (Fig 3D).

Considering the forthcoming amphetamine challenges, in the rats initially protected with pentadecapeptide BPC 157 at the time of the first application of amphetamine, the stereotyped behavior remained to be attenuated through all additional amphetamine applications. Moreover, in these days, a significant attenuation

appeared also in the rats that had been treated with the lower dose of the pentadecapeptide BPC 157 at the time of the first amphetamine application. To indicate, at some periods, the otherwise inescapable stereotyped behavior following amphetamine, was completely absent.

Stereotyped behavior score was decreased; on d 2 at BPC 157 10 µg during 5-40 min, 70-80 min, and

105–120 min and at 10 ng during 5–40 min and 75–80 min (Fig 3A); on d 3 at BPC 157 10  $\mu$ g during 5–15 min, 25–65 min, 95–120 min and at 10 ng during 5–10 min, 25–65 min, and 105–120 min (Fig 3B); on d 4 at BPC 157 10  $\mu$ g during 5–10 min and 65–120 min and at 10 ng during 5–10 min, 65 min, and 85–120 min (Fig 3C); on d 5 at BPC 157 10  $\mu$ g 5–40 min and 60–120 min and at 10 ng during 5–15 min and 60–110 min (Fig 3D).

**Heightened startle response** On d 2, paradoxically with respect to stereotyped behavior (exaggerated response), the reaction was rather attenuated (score 2 between 5–45 min after amphetamine) than exaggerated (ie. lack of normal reaction at the earliest interval, Fig 3E). This may suggest that heightened startle response was a special reaction, with its own amphetamine-background, at least partly different from stereotyped behavior. On d 3 the maximal reaction was shifted toward the earlier intervals (score 3 between 10–55 min, score 2 at 5 min, and 60–105 min, Fig 3F). Thus, reaction seemed to be more rapid than before. Probably related to suggested limited capacity for reacting with heightened startle response, normal behavior not seen after heightened startle response after first application of amphetamine, in these animals reappeared before the end of the observation period. After this time, on d 4 and d 5, the reaction toward amphetamine appeared to be sharply lessened, despite continuous applications (Fig 3G, 3H). On d 4 rats exhibited heightened startle response only at the earliest interval<sup>[10–25]</sup>, while on d 5, they showed no increased excitability as they did before. Thus, at the end of the conditioning period, in rats conditioned by repeated applications of amphetamine, an otherwise expected raised excitability was lacking. Of note, this is along with findings noted in stereotyped behavior assessment.

It should be noted that on d 5 all animals showed no increased excitability. However, the pentadecapeptide BPC 157-treated rats showed no increased excitability for an apparently longer period; on d 2 at BPC 157 10  $\mu$ g during 5–50 min and 65–120 min and at 10 ng during 5–40 min (Fig 3E); on d 3 at BPC 157 10  $\mu$ g and 10 ng during 5–105 min (Fig 3F) and on d 4 at both dosages during 10–20 min and 60–120 min (Fig 3G).

### Challenging period

**Stereotyped behavior** Rats received no further amphetamine challenge till d 8, and thereafter on d 16, and finally, on d 46. Confronted with the intermittent amphetamine administration, after a withdrawal period,

on d 8, 16, and 46, the intensity of stereotyped behavior was evidently decreased when compared with disturbance originally seen in amphetamine rats on d 1. However, in relation to the events noted at the end of the conditioning period (ie, d 5), on d 8 and d 16 stereotyped behavior was more prominent (behavior scored as 3 was between 25–110 min (on d 8, Fig 4A), or even 20–120 min (on d 16, Fig 4B). Interestingly, a further attenuation occurred at d 46, at the time of the application of the third challenge (Fig 4C). As mentioned above, although no stereotyped behavior of the lowest score (score 1) was noted, the disturbances characterized as score 2 were present through all the observation period, without otherwise expected raise.

In pentadecapeptide BPC 157 treated-rats, even in these changed circumstances, ie, withdrawal period, and thereafter, intermittent amphetamine challenges (d 8, 16, and 46) the stereotyped behavior remained to be attenuated; on d 8 at BPC 157 10  $\mu$ g during 5–120 min and at 10 ng during 10–15 min, 25–35 min, and 65–110 min (Fig 4A); on d 16 at BPC 157 10  $\mu$ g during 5–10 min and 20–120 min and at 10 ng during 5 min and 20–25 min (Fig 4B); on d 46 at BPC 157 10  $\mu$ g during 5–10 min and 25–120 min and at 10 ng during 5–10 min (Fig 4C). Unlike control-amphetamine rats, like in the conditioning period following initial application, in pentadecapeptide BPC 157 treated-rats no stereotyped behavior could be seen during some periods. Thus, pentadecapeptide BPC 157, given with the first initial amphetamine administration, affords a long-lasting protection against forthcoming amphetamine applications. In conclusion, since the stereotyped behavior was markedly attenuated in pentadecapeptide BPC 157 treated-rats regardless amphetamine given acutely, by continuous administrations during conditioning period, or by intermittent applications in subsequent challenging period, this effect may be hardly accidental.

**Heightened startle response** After a withdrawal period, amphetamine was given as an intermittent regimen (d 8, 16, and 46). Considering the effect of restarted amphetamine application, in relation to the disturbance originally seen in amphetamine rats on d 1, the intensity was evidently decreased on d 8. However, in relation to the events noted at the end of the conditioning period (ie, d 5), a heightened startle response reappeared, being present till the end of the observation (score 2 between 45–120 min, Fig 4D). Interestingly, at the more delayed challenge, on d 16, an increment was further seen (score 3 between 20–40

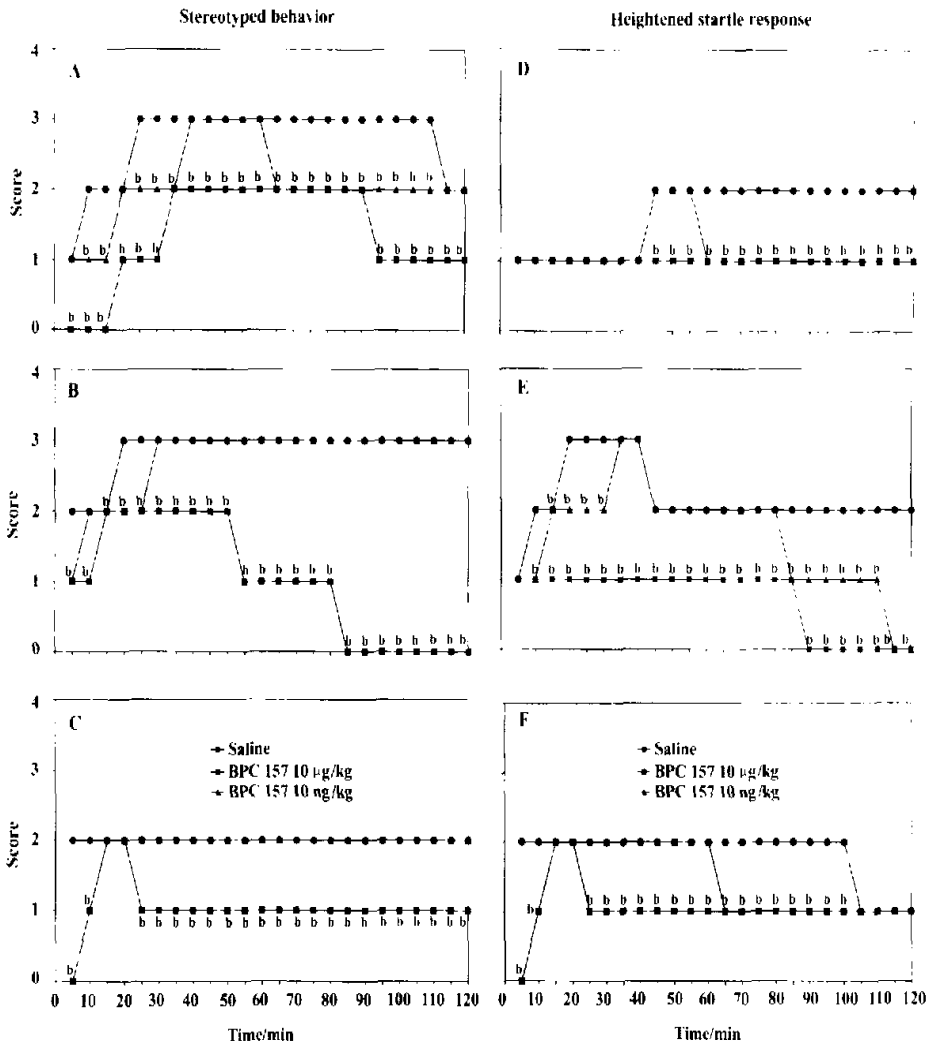


Fig 4. Effect of pentadecapeptide BPC 157 10 µg/kg ip or 10 ng/kg on amphetamine disturbances during challenging period with intermittent amphetamine application after withdrawal period. A, D; d 8; B, E; d 16; C, F; d 46.  $n = 6$ .  $^bP < 0.05$  vs saline.

min, score 2 between 10 – 15 min, and 45 – 120 min, Fig 4E). Normal behavior was lacking, except to the earliest interval (a finding already noticed after initial first amphetamine application). On the final day (d 46), this increment was still present, even the maximal intensity was longer (score 2 between 5 – 95 min), but the normal values, besides the earliest interval (ie, at 5 min), appeared before the end of the experimental period (100 – 120 min, Fig 4F). Confronted with the intermittent amphetamine administration, after a withdrawal period, the heightened startle response remained to be attenuated (at d 8, 16, and 46). This was consistent with the attenuation of stereotyped behavior seen in these rats. Pentadecapeptide BPC 157-treated

rats showed no increased excitability through all the observation period; on d 8 (Fig 4D) at BPC 157 10 µg during 45 – 120 min and at 10 ng during 60 – 120 min; on d 16 (Fig 4E) at BPC 157 10 µg during 10 – 120 min and at 10 ng at 10 min, during 20 – 30 min and 85 – 120 min; on d 46 (Fig 4F) at BPC 157 10 µg during 5 – 10 min and 25 – 95 min and at 10 ng during 5 – 10 min and 65 – 95 min.

Thus, pentadecapeptide BPC 157, given with the first initial amphetamine administration, affords a long lasting protection against heightened startle response that otherwise follows forthcoming amphetamine applications, regardless amphetamine given acutely, by continuous administrations during conditioning period, or by

intermittent applications in subsequent challenging period. Since the stereotyped behavior was also markedly attenuated by pentadecapeptide BPC 157, and considering the heightened startle response, this effect may indicate a general effect on amphetamine disturbances.

## DISCUSSION

The focus was on possible significance of a gastric pentadecapeptide BPC 157 for mitigation of amphetamine harmful effect. Recently, it was shown that gastric pentadecapeptide BPC 157 had a beneficial effect opposing acute amphetamine; it reduced disturbances development (ie, co-administered with amphetamine) as well as it reversed already developed disturbances (given at the time of the raised stereotyped behavior)<sup>(10)</sup>. Now, this study showed a complex influence of this pentadecapeptide on the chronic amphetamine application in rats. This may be important since chronic amphetamine application produces changes at least different from those seen after an acute single challenge<sup>(5-9)</sup>.

Amphetamine was given once daily throughout the five subsequent days of conditioning period (d 1 - d 5), and thereafter, as three late challenges at suitable time intervals (on d 8, 16, and 46). In general, in relation to the used initial-single/continuous/intermittent amphetamine application (s)-regimen<sup>(5-9)</sup>, the changes in stereotyped behavior and heightened startle response, increment or decrement, noted in control amphetamine rats, are in line with changes commonly referred in chronic amphetamine studies as tolerance and reverse tolerance<sup>(5-9)</sup>, dependent on the used protocol (dose, continuous/intermittent application) of the amphetamine and tested pattern(s) in given species. Thus, an initial mitigation of the amphetamine disturbances, ie, less grade of stereotyped behavior, without increased excitability, noted at the end of the conditioning period is compatible with assumption that the rats after five subsequent applications of the quite high dose of the amphetamine became temporary tolerant. As indicated, the used dose of amphetamine (10 mg/kg, ip) produced a profound depletion of catecholamines stores<sup>(6)</sup>, that fairly correlates with our results at the end of the conditioning with continuous amphetamine daily applications. Likewise, the reappearance of both more prominent stereotyped behavior and heightened startle response upon late amphetamine challenges is consistent with reported "reverse tolerance"<sup>(5-9)</sup> in chronic amphetamine treated-rats.

On the other hand, in chronic amphetamine treated-rats initially protected by a single application of pentadecapeptide BPC 157, the otherwise consistent amphetamine disturbances were regularly mitigated. Supportingly, this was seen on both disturbances, stereotyped behavior and heightened startle response. Likewise, this was backed by different amphetamine regimens through experimental periods: continuous daily amphetamine administrations (conditioning period) and after a withdrawal period, intermittent amphetamine applications on d 8, 16, and 46 (subsequent challenging period). This is in keeping with an essential role, and an immediate interference with amphetamine activity, recently reported for this pentadecapeptide BPC 157 in failure of dopamine system function<sup>(10)</sup>.

Pentadecapeptide BPC 157 was given only with first application of amphetamine. Therefore, considering that conditioning probably has little importance for postponed effect of amphetamine<sup>(8)</sup>, all of the changes responsible for further development should be already related to the first amphetamine application. Theoretically, if this essential change triggering all of the chain of events with long lasting consequences was initially either prevented, or at least, attenuated, then, otherwise inescapable negative events would be fully mitigated, even by very late period. This is fully supported by the described beneficial effects of  $\mu\text{g}$ -regimen on stereotyped behavior and heightened startle response, and ng-regimen on heightened startle response evidenced after both initial first amphetamine application, and thereafter through subsequent conditioning period with daily continuous administrations and challenging period with later intermitted applications. Based on these data, some effects on the initial events should be supposed, even when the statistical significance is initially not reached. For instance, in stereotyped behavior assessment, an attenuating effect of ng-regimen on stereotyped behavior appeared during the conditioning period and later, at the time when amphetamine challenge-postponed disturbances occurred, that would be hardly possible without assuming some effect on the initial triggering event. Therefore, by whatever mechanism, pentadecapeptide BPC 157 inhibited amphetamine induced-stereotyped behavior appears to be rather specific.

Furthermore, to explain this long lasting effect of pentadecapeptide BPC 157 on amphetamine disturbances, several considerations should be emphasized. Release of dopamine from nerve terminals and subsequent activation



of dopamine receptors in the striatum<sup>(2, 3, 5-9, 29)</sup> is implicated in amphetamine induced-stereotyped behavior. Therefore, it is likely that an enhanced stereotyped behavioral response to amphetamine chronically applied, is due to effect of this drug on the pre- or post-dopaminergic synapse in this region of the brain, and concept of the increasing receptor sensitivity with chronic stimulant administration<sup>(8)</sup> is commonly proposed. On the other hand, an administration of a dopamine receptor antagonist, haloperidol, may lead to the similar consequences; an over-increased amphetamine activity (climbing behavior)<sup>(10)</sup> (ie, a delayed result of striatal dopamine receptors up-regulation following dopamine blockage)<sup>(10)</sup>. To emphasize an antagonizing effect of pentadecapeptide BPC 157 in both conditions (ie, attenuation of haloperidol/amphetamine-climbing behavior, long lasting attenuation of amphetamine-stereotyped behavior and heightened startle response), it should be noted that in both experiments carried out in the previous<sup>(10)</sup> and present study, pentadecapeptide BPC 157 was given simultaneously with the initial, early challenge. Accordingly, pentadecapeptide BPC 157 opposing effect was assessed at the time of the postponed amphetamine applications (ie, days thereafter). Concerning the obvious differences between the initial challenges (ie, haloperidol<sup>(15)</sup> vs amphetamine<sup>(5-9)</sup> administration), it means that this pentadecapeptide BPC 157 effect may have a general significance. Thus, the mitigation of the amphetamine harmful effects in both acute and chronic amphetamine experiments was not related to any specific dopamine antagonistic activity (direct and/or indirect). Noteworthy, if this was the case, an additional potentiation of the haloperidol increasing effect on amphetamine climbing behavior had been obtained, unlike mentioned abolition<sup>(10)</sup>. Common mechanism(s) likely related to gastric pentadecapeptide BPC 157 supported also the evidence that it antagonized catalepsy and somatosensory disorientation induced by dopamine antagonists (haloperidol, fluphenazine, clozapine, and sulpiride)<sup>(11)</sup>, as well as MPTP- or reserpine-disturbances<sup>(12)</sup>. The same  $\mu\text{g}$ - and  $\text{ng}$ -regimen of pentadecapeptide BPC 157 were used in present and previous studies<sup>(10-12)</sup>. Thus, taking these data together, it seems that this pentadecapeptide has a modulatory effect on dopamine system.

Obviously, the noted salutary effect of the pentadecapeptide BPC 157 could be hardly accidental, and a special interaction with striatal dopamine receptors should be likely suggested. In analogy, since the effect

of haloperidol-induced dopamine receptors up-regulation appeared not before two days following haloperidol treatment<sup>(15)</sup>, in addition to an interference with very early events leading to later amphetamine supersensitivity appearance, the pentadecapeptide BPC 157 could have a correspondingly prolonged salutary action. Although this is probably not direct related, it should be noted that this gastric pentadecapeptide is stable in gastric juice at least for 24 h<sup>(35)</sup>. It has mucosal protective<sup>(11, 16, 24, 31-34)</sup> wound healing<sup>(21-26, 28)</sup>, and anti-inflammatory effect<sup>(27)</sup>, interacting with NO<sup>(22)</sup> and somatosensory neurons system<sup>(23)</sup>. It is probably more than a simple coincidence that a small dose of amphetamine (0.8 mg/kg, ip) acutely did not reduce the concentration of noradrenaline in either brain or heart (the reduction appeared after chronic treatment), but it produced a 50 % reduction in the noradrenaline content in the stomach in rat<sup>(6)</sup>. Dopamine opposing agents, receptor blockers (haloperidol, sulpiride, and domperidone)<sup>(11, 19, 36)</sup>, acting both centrally or peripherally, nigrostriatal dopamine neurotoxin MPTP, and of dopaminergic intraneuron granules depletor reserpine<sup>(12)</sup>, in addition to central disturbance produced gastric lesion, that were both attenuated by pentadecapeptide BPC 157 application. For instance, haloperidol-produced prominent gastric lesion in rat<sup>(11, 36)</sup> and mouse<sup>(19)</sup>, while dopamine agonists (ie, bromocriptine, amantadine)<sup>(19, 36)</sup> and gastric pentadecapeptide BPC 157 antagonized these lesions, unlike other antiulcer agents<sup>(19)</sup>. Besides, a particular interaction of gastric pentadecapeptide BPC 157 with central dopamine system was also shown in other experimental models (ie, protection stress ulcers)<sup>(21)</sup>. Likewise, a considerable part of evidence about interaction of gut peptides with dopamine system arised from gastric mucosal protection studies<sup>(11)</sup>.

In summary, a persistent opposing effect of the pentadecapeptide BPC 157 throughout the chronic exposure to amphetamine in rats, described in the present study is compatible with its previously described effects<sup>(11-13)</sup>. Therefore, a particular modulatory role may be suggested. Finally, particularly with respect to indicated GABA/dopamine systems interactions<sup>(15, 16)</sup>, besides an anxiolytic effect<sup>(17)</sup>, a possible analogy for a prolonged activity could be noted; a lack of tolerance development was demonstrated in tolerance studies, while residual anticonvulsive activity was prolonged, and physical dependence/withdrawal hallmark was postponed when pentadecapeptide BPC 157 was given simul-

taneously with diazepam<sup>14</sup>.

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### 十五肽 BPC 157 减弱慢性苯丙胺诱导的行为障碍

R96 A  
关键词 BPC 157; 苯丙胺; 刻板行为

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