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The Effect of a Novel Pentadecapeptide BPC 157 on Development of Tolerance and Physical Dependence Following Repeated Administration of Diazepam

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Abstract

A novel gastric pentadecapeptide BPC 157 with different beneficial activities and anticonvulsant effect interacting with GABAergic system could improve diazepam efficacy coadministered (10 μg/kg, 10 ng/kg i.p.) with diazepam (5.0 mg/kg i.p.) twice daily for 10 days, since diazepam chronic medication would otherwise predispose for diazepam- tolerance/withdrawal development (shorter latency to convulsion after convulsant)). In diazepam chronically treated mice, it attenuated diazepam tolerance (provoked by later acute administration of diazepam together with convulsant) and postponed physical dependence/withdrawal effects (provoked by later administration of isoniazid). In tolerance assay, at 42h after the end of conditioning regimen, shorter preconvulsive latencies than in healthy (non-diazepam conditioned) mice following isoniazid (800 mg/kg i.p.) (as hallmark of tolerance) were observed if diazepam (5.0 mg/kg i.p.) was again given acutely to mice previously conditioned with diazepam alone (use of picrotoxin 3.0 mg/kg i.p., as convulsant, with acute application of diazepam in previously diazepam conditioned mice did not lead to tolerance hallmark). This was completely avoided in diazepam+BPC 157 10 μg or diazepam+BPC 157 10 ng chronically treated animals. In physical dependence assay (isoniazid challenge assessed at 6, 14, 42 and 72h after conditioning medication), when compared to diazepam non-conditioned healthy mice, in diazepam conditioned mice residual anticonvulsive activity was not present already at the earliest post-conditioning interval (i.e., not different latency to isoniazid-convulsions), whereas shorter preconvulsive latencies (as physical dependence/withdrawal hallmark) were noted in diazepam conditioned mice following isoniazid challenge at 42 h and at 72 h after end of conditioning treatment. In diazepam+BPC 157 10 μg- conditioned mice, a residual anticonvulsive activity (i.e., longer latency to isoniazid convulsion) was noted at 6 h post-conditioning, whereas shorter preconvulsive latencies appeared only at 72 h-post-conditioning period. In conclusion, taken together these data (lack of tolerance development (tolerance studies), prolonged residual anticonvulsive activity, and postponed physical dependence/withdrawal hallmark in diazepam+BPC 157 chronically treated mice) with common benzodiazepines tolerance/withdrawal knowledge, it could be speculated that BPC 157 acts favoring the natural homeostasis of the GABA receptor complex as well as enhancing the GABAergic transmission, and having a mechanism at least partly different from those involved in diazepam tolerance/withdrawal, it may be likely used in further therapy of diazepam tolerance and withdrawal.

Key Words: benzodiazepines, tolerance, physical dependence/withdrawal, pentadecapeptide BPC 157, convulsions, isoniazid, $GABA_A$ receptor

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Introduction

Because of the physiologic significance of gut peptides and possible therapeutic application, the discovery of so far unknown peptides, their original structure and saving actions, have received considerable attention. As previously pointed out, we identified a new human gastric juice protein with mucosal protective properties and a huge range of organoprotective effects, M.W. 40,000 (determined by gel chromatography), code-named BPC. The regular procedures, i.e., controlled dialysis of gastric juice, lyophylisation, chromatographic separation on weak anion exchange resin (DEAE), cation exchange resin (S), following by gel chromatography and finally HPLC were applied. In line with this, a 15 amino acid fragment (BPC 157), with particular aminoacid sequence (Gly Glu Pro Pro Pro Gly Lys Pro Ala Asp Asp Ala Gly Leu Val), M.W. 1,419, and apparently no sequence homology to known gut peptides, thought to be essential for activity of an entire peptide, was characterized and synthesized (1-20). Considering the origin, the first focus in the investigation of the pentadecapeptide BPC 157 was on its prominent salutary activity on the various gastrointestinal injuries induced by diverse ulcerogens (1, 4-12, 20-24, 26-28), suggesting that the noted beneficial effects include apparently the entire gastrointestinal tract. Likewise, it was claimed to have beneficial effects even outside gastrointestinal tract (3) injuries in rats or reduction of acute and chronic inflammation (11) and inflammatory mediators release (26-28), and increased wound healing and fracture healing (1, 7, 13, 16, 19, 20). Intriguingly, this includes also heart protection, following hypoxic and reoxygenation injury in the isolated guinea pig heart (1, 29).

In further analysis using a modification of the pentadecapeptide BPC 157 salutary effect by particular challengers (somatosensory neurons depletion by neurotoxin capsaicin (6), blockade or stimulation of NO-synthesis (10, 14) or dopaminergic and/or catecholaminergic systems (8), inhibition of prostaglandins synthesis (6, 11)) as a hallmark, besides others, an interaction with dopaminergic and/or catecholaminergic systems, particularly central was suggested (8). Although the binding studies were so far unable to show any binding to dopamine receptors, this was a basis for recent demonstration that the pentadecapeptide BPC 157, although it has no influence on gross behavior in normal animals, may block stereotypy produced by the dopamine agonist amphetamine (17). Moreover, it blocks also increased effect of amphetamine following dopamine antagonist haloperidol application (i.e., climbing behavior) in mice (17). Likewise, it antagonizes neuroleptics induced catalepsy (18). Along with this, it was previously shown that pentadecapeptide BPC 157 may improve motor disturbances induced by neurotoxin 1-methyl-4-pheny 1-1, 2, 3, 6-tetra-hydropyridine (MPTP), a Parkinsongenic neurotoxin, affecting nigrostriatal dopamine (30), or reserpine, a depletor of dopaminergic intraneuron granules (30).

Since GABA-ergic transmission is longly implicated in the regulation of dopamine-mediated events within the extrapyramidal systems, and behaviors dependent on striatal functions (catalepsy, stereotypes) (31, 32), it was interesting to see the effect of pentadecapeptide BPC 157 on GABA-system disturbances, such as diazepam induced tolerance/withdrawal (33-37). Diazepam tolerance was evaluated in a well-known model of diazepam-conditioned mice, compared with the corresponding (non-diazepam conditioned) healthy animals, as a shortening of the latency to convulsions in diazepam chronically treated mice after convulsant challenge if diazepam was given again, as an acute administration, together with an convulsant (i.e., isoniazid) at the suitable periods post-conditioning (36). Physical dependence commonly studied in similar models as an increased sensitivity to convulsant challenge (33-37), was assessed in diazepam chronically treated mice at different time points after discontinuation of diazepam conditioning as a decrease in the latency to convulsions induced by convulsant challenge (36). The development of tolerance (for review see, i.e., 38) and physical dependence (for review see, i.e., 39) are among the most serious side-effects of benzodiazepine therapy. Important to indicate, pentadecapeptide BPC 157 has anticonvulsant activity against different challenges (1).

Materials and Methods

Drugs

BPC 157. Preparation of the Peptide

The pentadecapeptide BPC 157 (GlyGluProProProGlyLysProAlaAspAspAlaGlyLeuVal), MW 1,419 is a partial sequence of human gastric juice protein BPC, freely soluble in water at pH 7.0 and in saline. It was prepared by solid phase peptide synthesis using t-BOC-Val loaded HYCRAM® polymer carrier (ORPEGEN, GmB, Heidelberg). The t-BOC amino acids were coupled in consecutive steps using diisopropylcarodimide/1-hydroxybenzentirazole reagent for activation. After the sequence completion the partially protected peptide was cleaved from polymeric carrier by hydrogenation and purified on polymeric carrier and purified on silicagel column, all protecting groups were removed with trifluoroacetic acid and the peptide finally purified on

silicagel column, all protecting groups were removed with trifluoacetic acid and the peptide finally purified by RP HPLC. Peptide with 99% (HPLC) purity (1-des-Gly peptide as impurity) was used (1-20).

Diazepam (Belupo, Croatia) and isoniazid (Pliva, Croatia) were dissolved in distilled water (containing 0.1% Tween), picrotoxin (Sigma, USA) was dissolved in saline.

Animals

Male NMRI mice, weighing 22-24 g, were used for all the experiments. Mice were housed in groups of 10 and maintained on a 12-hour light cycle (lights on at 7.00 a.m.) with free access to food and water.

Repeated Drug Treatment

The dose of diazepam (5.0 mg/kg i.p., twice daily at 8.00 a.m. and 4.00 p.m. for 10 consecutive days) was chosen on the basis of its anticonvulsant activity and corresponds to 2 times the ED50 against convulsions induced by isoniazid (p.o.) (36). The control groups received only repeated treatment with diazepam (5.0 mg/kg i.p.) and/or saline (5.0 ml/kg i. p.). Pentadecapeptide BPC 157 (10 ng/kg or 10 μg/kg i.p.) was given simultaneously with diazepam.

Anticonvulsant Activity

Tolerance was assessed in the conditioned animals by a decrease in anticonvulsant activity of diazepam against isoniazid- and picrotoxin-induced convulsions evidenced as a shortening of the latency to the appearance of the first convulsion compared with the corresponding (non-diazepam conditioned) healthy mice. The latency to the first tonic-clonic convulsion was measured following convulsant (isoniazid or picrotoxin) administration, at 42 h after discontinuation of the conditioning medication when diazepam (5.0 mg/kg i.p.) was given either simultaneously with isoniazid (800 mg/kg i.p.) or 30 min before picrotoxin (3.0 mg/kg i.p.) (see Fig. 1).

The intrinsic anticonvulsant activity of BPC 157 (10 ng/kg and 10 μg/kg i.p.) was evidenced in non-conditioned naive animals. Isoniazid (800 mg/kg i.p.) was administered simultaneously with BPC 157, whereas vehicle was given to the control group. Diazepam (5.0 mg/kg i.p.) basal anticonvulsant activity was in the same way studied.

A shortening of the latency to isoniazid-induced convulsions in the conditioned mice, compared with the (non-diazepam conditioned) healthy animals, was taken as an index of physical dependence. It was evaluated at different intervals (6, 14, 42, 72 h) after withdrawal of BPC 157 and/or diazepam repeated

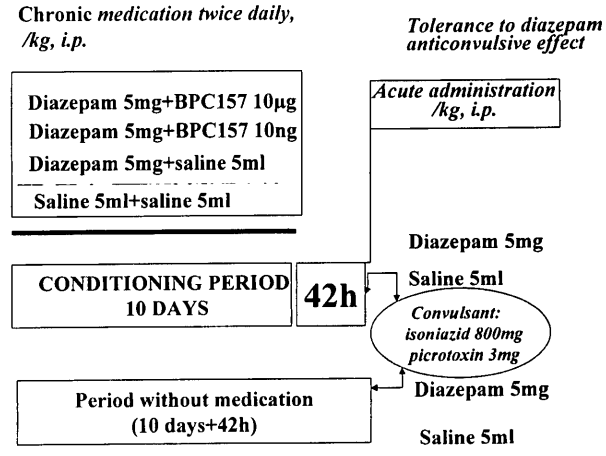

Fig. 1. Tolerance was assessed in the conditioned animals by a decrease in anticonvulsant activity of diazepam (applied as an acute application after the end of chronic medication) against isoniazid (see Fig. 4A)- and picrotoxin-induced convulsions, evidenced as a shortening of the latency to the appearance of the first convulsion compared with the corresponding healthy (non-diazepam conditioned) mice (see Fig. 4B). The dose of diazepam (5.0 mg/kg i.p., twice daily at 8.00 a.m. and 4.00 p.m. for 10 consecutive days) (chosen on the basis of its anticonvulsant activity corresponding to 2 times the ED50 against convulsions induced by isoniazid (p.o.) (36)) was used for conditioning regimen, the mice received only repeated treatment with diazepam and/or saline (5.0 ml/kg i.p.). Pentadecapeptide BPC 157 (10 ng/kg or 10 μg/kg i.p.) was given simultaneously with diazepam. The latency to the first tonic-clonic convulsion was measured following convulsant (isoniazid or picrotoxin) administration, at 42 h after discontinuation of the conditioning medication when diazepam (5.0 mg/kg i.p.) was given either simultaneously with isoniazid (800 mg/kg i.p.) or 30 min before picrotoxin (3.0 mg/kg i.p.)(since the anticonvulsant activity of diazepam, given acutely in conditioned mice, was not changed to any extent in diazepam conditioned mice when compared to diazepam-non-conditioned mice, these data were not specifically listed).

treatment. Mice were given isoniazid (800 mg/kg i. p.) and the latency to the first convulsion was measured as described above (see Fig. 2).

Statistical Analysis

The effects of repeated treatments were evaluated statistically by comparing the convulsion latencies observed in mice treated with vehicle with mice repeatedly treated with drugs using ANOVA and Kruskal-Wallis ANOVA and postcomparison test (Tukey honest significant difference test).

Results

Isoniazid/Picrotoxin/Pentadecapeptide BPC 157

BPC 157 showed a significant intrinsic anticonvulsant activity as shown by an increased latency to isoniazid-induced convulsions in naive

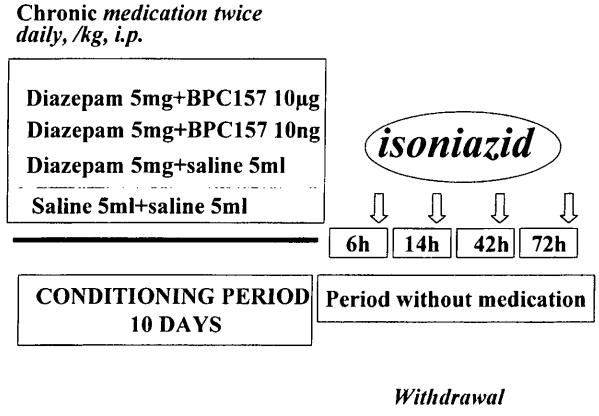

Fig. 2. A shortening of the latency to isoniazid-induced convulsions in the conditioned mice, compared with the healthy (non-diazepam-conditioned) animals, was taken as an index of physical dependence/withdrawal. It was evaluated at different intervals (6, 14, 42, 72 h) after the end of conditioning protocol (see Fig. 1 for details), when mice were given isoniazid (800 mg/kg i.p.) and the latency to the first convulsion was measured.

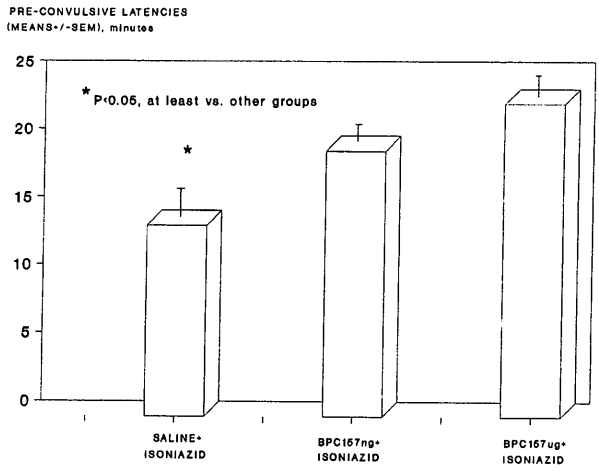

Fig. 3. Anticonvulsant effect of pentadecapeptide BPC 157 (10 µg or 10 ng/kg i.p.) given simultaneously with isoniazid (800 mg/kg i.p.) to naive mice. 10-15 mice per each experimental group.

mice (Fig. 3), or as a lack of convulsions in picrotoxin-challenged animals.

Thus, the possibility that the limitation in diazepam efficacy (tolerance to anticonvulsant activity and/or withdrawal effect) could be in this way avoided, appears to be justified.

Tolerance to Anticonvulsant Activities

Isoniazid: In the animals repeatedly treated with diazepam alone, for 10 subsequent days, twice daily, the forthcoming acute administration of diazepam, given simultaneously with isoniazid at 42 h post-conditioning was significantly less protective (preconvulsive latencies shorter) against isoniazid-convulsions, contrasting with the effect in naive (diazepam-non-conditioned) mice or mice treated with saline for 10 days (Fig. 4).

In distinction from the above described effect in the animals repeatedly treated with diazepam alone, in the groups treated for 10 days with a combination of diazepam and BPC 157 (coadministered in dosages of 10 µg/kg or 10 ng/kg i.p.), the same acute application of diazepam given at 42 h post-conditioning, protected apparently better against isoniazid-convulsions (Fig. 4.). Since latencies were generally longer in diazepam+BPC 157 µg and diazepam+BPC 157 ng than in animals repeatedly treated with diazepam alone, i.e., without pentadecapeptide BPC 157 co-treatment, and not different from those noted in mice without diazepam conditioning (mice repeatedly treated with saline), it seems likely that the tolerance to anticonvulsive effect of diazepam is not present in diazepam+BPC 157 chronically treated mice.

Picrotoxin: Picrotoxin challenge at 3.0 mg/kg induced convulsions in all naive mice, but not in mice repeatedly treated with BPC 157 and diazepam as well as in mice repeatedly treated with diazepam alone. Unlike diazepam chronically treated mice challenged with an acute administration of diazepam and convulsant isoniazid, the picrotoxin-convulsions were completely absent in diazepam chronically treated animals when diazepam (5.0 mg/kg i.p.) was administered again as an acute administration 30 minutes before the picrotoxin challenge. Thus, assessed with picrotoxin-challenge, the tolerance to anticonvulsive effect of diazepam in chronically diazepam treated mice is not present.

Withdrawal Effects

Conditioning with diazepam alone: Applied in naive (diazepam-non-conditioned) mice, diazepam (5.0 mg/kg i.p.) had a strong protective effect against isoniazid (i.e., compared with saline-treated mice, it had markedly prolonged latency to isoniazid convulsion) (Fig. 4B). However, the latency to convulsion of isoniazid will be changed in diazepam chronically treated mice, as could be seen by assessing the shortening of latency to this convulsant challenge. Considering the latency to convulsion of isoniazid challenge in diazepam chronically treated mice, when isoniazid was given at the suitable periods (i.e., at 6, 14, 42 or 72 h) after the end of chronic diazepam regimen in the diazepam-conditioned mice, time dependent changes in latencies to convulsions were consistently observed (Fig. 5). Firstly, a lack of anticonvulsant effect of diazepam seems to be present in chronically diazepam treated mice already at the first observation period, since isoniazid challenge at the 6 h interval showed preconvulsive latencies similar

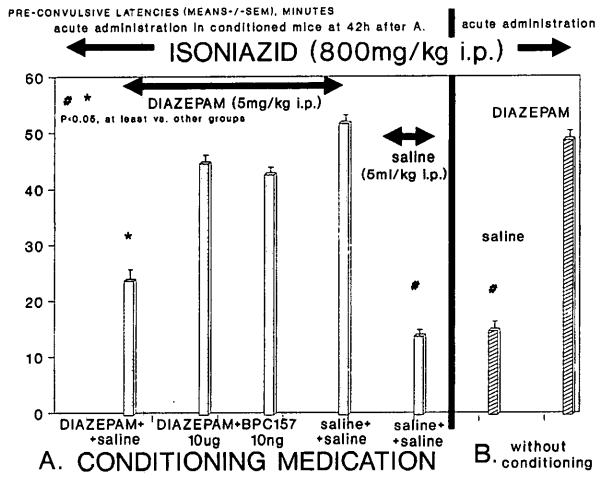

Fig. 4. Tolerance to diazepam anticonvulsive effect. Conditioning medication (diazepam 5 mg/kg, BPC 157 10 μg or 10 ng/kg, saline 5 ml/kg, given i.p. in various combinations, for 10 subsequent days) indicated in the bars. Diazepam (5 mg/kg i.p.) or saline (5 ml/kg i.p.) was applied simultaneously with isoniazid (800 mg/kg i.p.) in either conditioned (42 h after discontinuation of conditioning medication) or in naive (diazepam-non-conditioned) mice. 10-15 mice per each experimental group.

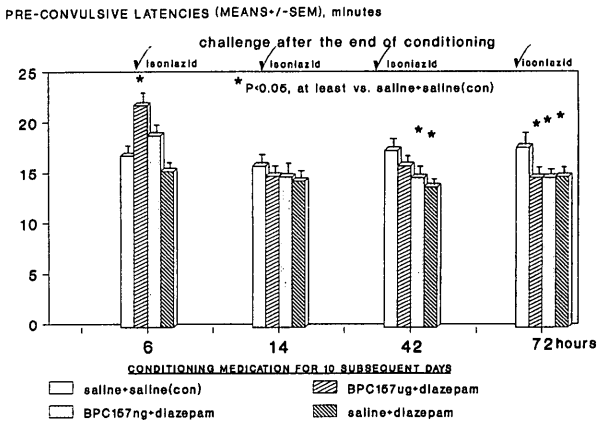

Fig. 5. Withdrawal effect - physical effect of diazepam. Conditioning medication (diazepam 5 mg/kg, BPC 157 10 μg or 10 ng/kg, saline 5 ml/kg, given i.p. in various combinations, for 10 subsequent days). Isoniazid (800 mg/kg i.p.) was given at various periods after discontinuation of conditioning medication (6 h, 14 h, 42 h, 72 h) to conditioned mice. 10-15 mice per each experimental group.

for the mice that had been repeatedly treated with diazepam like as with saline. In the forthcoming period (6-14 h post-conditioning), no difference in the reactivity of the animals to isoniazid challenge may be noted regardless they had been previously conditioned with saline or diazepam (i.e., a persistent lack of anticonvulsant effect of diazepam), hence these latencies remained substantially the same until at 14 h-assessment. But, during the next period (14h-42h post-conditioning) a substantial change could be suggested in the reactivity to isoniazid challenge: the animals that had been previously conditioned with diazepam became more vulnerable than mice chronically treated with saline, since at 42h-assessment the latencies to isoniazid convulsion in diazepam chronically treated mice decreased bellow the control values. The shorter latency to isoniazid convulsion than in corresponding controls indicates that the physical dependence/withdrawal is achieved in the diazepam chronically treated mice at 42 h following last diazepam application. Importantly, the shorter latency than otherwise remained substantially the same during the last observation period (42h-72h post-conditioning). Thus, it did not reach the control values also during next 30 hours, indicating that the withdrawal following the end of the chronic diazepam regimen is consistently present throughout this period (i.e., at 72 h-assessment following discontinuation of the conditioning regimen the latency were again noted to be shorter in mice conditioned with diazepam than in those conditioned with saline).

Conditioning with diazepam and pentadecapeptide BPC 157 coadministration: Applied alone, BPC 157 had a marked protective effect against isoniazid convulsions, in either 10 μg/kg or 10 ng/kg dose (Fig. 3). Compared with the mice conditioned with diazepam alone, the changes noted when isoniazid was applied latter at the mentioned intervals following discontinuation of the conditioning regimen, appeared to be markedly and dose-dependently postponed. A dose dependent salutary effect (i.e., longer latency to isoniazid convulsion) seems to be still present at the first interval (6 h) (mice repeatedly treated with diazepam + BPC 157 μg vs. mice repeatedly treated with saline, as well as vs. mice repeatedly treated with diazepam). The same tendency was present in mice repeatedly treated with diazepam and BPC 157 at 10 ng/kg, but this effect was not statistically significant. Hence, in animals conditioned with diazepam and μg-dose of the pentadecapeptide BPC 157, convulsive effect of isoniazid seems to be still depressed at least for first 6 hours (of note, since no similar effect could be noted in the mice conditioned with diazepam alone, this could be hardly due to residual activity of diazepam). In these diazepam + BPC 157 μg -mice, at later time point (14 h-assessment) the latencies were not significantly different from those observed in the group repeatedly treated with saline, but also at the next point, i.e. at 42 h assessment post-conditioning, where shorter latency (and physical dependence/withdrawal) were noted in animals conditioned with diazepam alone. However, at the last time point (72 h-post-conditioning) their latencies appeared shorter in comparison with the control values, indicating that the appearance of the withdrawal following end of chronic diazepam is postponed for

30 h when pentadecapeptide BPC 157 μg had been coadministered with diazepam. Noteworthy, in mice conditioned with diazepam and lower dose of the pentadecapeptide BPC 157, the course of the withdrawal development was similar to that noted in mice conditioned with diazepam alone.

Discussion

In the present study, tolerance to the anticonvulsant effect of diazepam was assessed by measuring the antagonism of convulsions in model involving dysfunction of the GABAergic system: isoniazid- or picrotoxin-induced clonic-tonic convulsions. Likewise, decrease in the latency to convulsions induced by isoniazid following discontinuation of repeated treatment with diazepam was taken as index of withdrawal (36). Thus, using a well known protocol, after repeated diazepam treatment for 10 subsequent days, the disturbed diazepam conditions were basically well established in the present investigation, and consistent with previous results in tolerance- and withdrawal-studies (33-37). Furthermore, the evidence that the latency for picrotoxin-convulsion, a non-competitive blocker of GABA-receptor chloride channels (40, 41), after acute diazepam application in diazepam chronically treated mice became not shorter than otherwise, unlike shorter latency for isoniazid-convulsion in chronically diazepam treated mice, is consistent with the suggestion that the tolerance could be developed for one convulsant (i.e., isoniazid), but not for an other (42) (i.e., picrotoxin) as well as that chloride channels are less affected after repeated diazepam treatment (42, 43). Therefore, the obtained evidence in mice chronically treated with diazepam and pentadecapeptide BPC 157 may be also indicative. Given simultaneously with the repeated diazepam treatment throughout 10 days-conditioned period, BPC 157 prolonged the otherwise shortened latency to isoniazid convulsion in mice chronically treated with diazepam in either experiment (acute diazepam challenge+isoniazid (tolerance studies), or isoniazid only (physical dependence/withdrawal studies)), thereby influencing both tolerance and withdrawal (the events, sharing the same essential background (i. e., withdrawal occurs as a result of tolerance (38))). This strongly suggests that this pentadecapeptide may affect the chain of events leading to tolerance and withdrawal development following prolonged administration of benzodiazepines. The way how this could happen, remains to be further clarified.

Theoretically, BPC 157 given simultaneously with repeated diazepam could act postsynaptically preventing the downregulation of the benzodiazepines receptors. This may be the case despite decreased response to chronic benzodiazepines does not appear to be consistently related to alterations in the number of affinity of receptors for benzodiazepines (i.e., 32, 43). In addition, BPC 157 may also reduce compensatory changes in other components of the ionophore GABAA receptor complex (for review see 37,38). However, so far no binding to GABAA receptor was demonstrated. Thus, several other factors should be considered in order to explain the beneficial effect of BPC 157 in both attenuating the development of tolerance as well as postponing the onset of physical crisis after discontinuation of repeated treatment with diazepam. Besides the molecular mechanism through which diazepam interacts with the receptor, an additional contribution of GAD (glutamic acid decarboxylase) specifically affected by isoniazid (i. e., inhibited GABA synthesis) (40, 41), the duration of action of BPC 157 and diazepam (ie., their intrinsic anticonvulsant activities) should be emphasized.

Since the BPC 157 anticonvulsant activity against isoniazid-induced convulsions was also evident in naive mice, BPC 157 may accordingly with general knowledge (i.e., 40, 41) also facilitate the GABA interaction with the GABA receptor site and/ or act at the GAD level, stimulating the synthesis of GABA or simply reducing the isoniazid inhibition of the enzyme. On the other hand, it is unlikely that BPC 157 may have an effect similar to that of diazepam, since if this was the case, it would be expected to increase the diazepam- tolerance/withdrawal negative effect (i.e., shorter latency to isoniazid-convulsion) rather than opposed them. Namely, prolonged exposure to benzodiazepines agonists (such as diazepam) shifts the responses of the whole spectrum of benzodiazepines ligands toward inverse agonist properties (i.e., anxiogenic, convulsant properties) (46). Considering that the diazepam tolerance/ withdrawal developed due to GABA-ergic failure, decrease GABA-ergic transmission (36), it may be that this pentadecapeptide may act paralelly, at least partly substitutes otherwise disturbed GABA-system function, particularly at the central sites (i.e., cortical), thought to be responsible for tolerance development, and thereby attenuating diazepam- tolerance/ withdrawal (43, 44, 47). For instance, in diazepam-tolerance studies, at 42 h after the end of the chronic administration of diazepam, the acute administration of diazepam has a similar anticonvulsive effect against isoniazid challenge as it had before only in the groups chronically treated with pentadecapeptide BPC 157 with diazepam, contrasting with apparently shorter latency of convulsion than otherwise in mice conditioned with diazepam but without pentadecapeptide BPC 157 co-application. Likewise, in diazepam-withdrawal studies, at 42 h after the end of the chronic administration of diazepam, physical dependence assessed by the shorter latency to isoniazid

convulsion than otherwise, was clearly noted in mice chronically treated with diazepam, but not if the mice had been chronically treated with 10 μg/kg pentadecapeptide BPC 157 together with diazepam. In these later animals, the shorter latency to isoniazid convulsion than otherwise (i.e., in non-conditioned mice) was postponed, and it was identified 30 h latter than in other mice chronically treated with diazepam (i.e., at 72 h post-conditioning). In this, for the suggested pentadecapeptide BPC 157-diazepam importance, of particular notition may be the evidence that in the withdrawal studies, where diazepam acute administration is lacking before isoniazid challenge the regimen with the lower dose of pentadecapeptide BPC 157 seems to be not effective, unlike tolerance-studies in chronically diazepam treated mice where acute diazepam administration was before isoniazid challenge, and the protocol with the lower dose was effective as well.

Finally, whatever the mechanism of the central action of this pentadecapeptide after its intraperitoneal application, behavioral effects of the peptides - given peripherally - are commonly thought to be an outward expression of specific cellular signals, most likely initiated at some visceral receptive relay of the central nervous system (48). Besides, there are few regions in the brain where the blood-brain barrier does not exist, the so called circumventricular organs, and here, some peptides act on specific peptide receptors to stimulate neuronal pathways within the brain (49). Likewise, in keeping with the generally known presence of the gut peptides in both brain and gut (50), the suggested presence of the pentadecapeptide BPC 157 not only in the stomach, also in the brain, although not fully defined (1), could possibly be responsible for the effect noted. However, like in the case of diazepam, when acute administration is not considered to be responsible for the effects obtained in chronically diazepam treated animals (42), it is unlikely that BPC 157 could be present at effective anticonvulsant dose 42 h after the last administration in diazepam+BPC 157-chronically treated mice, at the time of the acute challenge of diazepam together with isoniazid. This seems to be the case, although in withdrawal studies showed after 6 h a latency still longer in groups that had been chronically treated with diazepam + 10 μg/kg BPC 157 than in corresponding control, and despite stability of this pentadecapeptide stable in human gastric juice at least for 24 h (25)) and, therefore, another previously exerted effect should be hypothesized, e.g. activation of a second messenger amplification system (51). The latter could also explain the time- and dose-dependent course of the effect (51).

Finally, considering our previously reported effect of pentadecapeptide (17, 18) on behavior depending on striatal dopamine functions, the noted beneficial effect on diazepam-tolerance/withdrawal may be entirely expected. Namely, in support of the previously suggested involvement of catecholamines in benzodiazepines-tolerance/withdrawal (36), and GABA-ergic transmission in the regulation of dopamine mediated events within the extrapyramidal system, particularly behaviors dependent on striatal function (32), an analogy could be find between tolerance/withdrawal and previously investigated climbing behavior (17). Commonly, the focus was on the assessment of a late challenge (isoniazid (36) vs. amphetamine (17, 31) provoked over-effect (shortening of the latency of convulsion (36) vs. climbing (17, 31)), that appeared after a delayed period following disturbed conditions induced by an initial treatment (diazepam-GABA-system failure (36) vs. haloperidol-dopamine receptor blockade and subsequent increase of dopamine receptor number/sensitivity (31)); initial treatment and late challenge opposes each other (diazepam, GABA-mimetic agent vs. isoniazid, GABA-blocking agent (36, 40, 41); haloperidol dopamine receptor blocker vs. amphetamine, dopaminomimetic (17, 31)). Thus, considering the antagonizing effect of pentadecapeptide given with haloperidol on later climbing behavior induced by amphetamine challenge, it seems that an essentially same effect of pentadecapeptide BPC 157 (modulation of otherwise disturbed function) like in diazepam-tolerance/withdrawal model was already noted in dopamine-system disturbance (17). Moreover, the effect of BPC 157 could have been evident unrelatedly to the diazepam administration together with isoniazid, because benzodiazepines only facilitate the binding of GABA to its receptor site and, hence, they would have no effect if there is no GABA as it happens after isoniazid blockade of GAD (40).

In conclusion, taken together the evidence in diazepam tolerance/withdrawal studies (i.e., 33, 37), it could be speculated that BPC 157 acts favoring the natural homeostasis of the GABA receptor complex as well as enhancing the GABAergic transmission. However, regardless the precise mechanism of the pentadecapeptide BPC 157 salutary effect (i.e., lack of tolerance development (tolerance studies), prolonged residual anticonvulsive activity, and postponed physical dependence/withdrawal hallmark in diazepam+BPC 157 chronically treated mice) remains to be fully elucidated, it could be at least partly different from those involved in diazepam tolerance/withdrawal, and may be likely used in further therapy of diazepam tolerance and withdrawal.

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