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ORIGINAL ARTICLE

Class side effects: decreased pressure in the lower oesophageal and the pyloric sphincters after the administration of dopamine antagonists, neuroleptics, anti-emetics, L-NAME, pentadecapeptide BPC 157 and L-arginine

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Abstract The ulcerogenic potential of dopamine antagonists and L-NAME in rats provides unresolved issues of anti-emetic neuroleptic application in both patients and experimental studies. Therefore, in a 1-week study, we examined the pressures within the lower oesophageal and the pyloric sphincters in rats [assessed manometrically (cm H_2O)] after dopamine neuroleptics/prokinetics, L-NAME, L-arginine and stable gastric pentadecapeptide BPC 157 were administered alone and/or in combination. Medication (/kg) was given once daily intraperitoneally throughout the 7 days, with the last dose at 24 h before pressure assessment. Given as individual agents to healthy rats, all dopamine antagonists (central [haloperidol (6.25 mg, 16 mg, 25 mg), fluphenazine (5 mg), levomepromazine (50 mg), chlorpromazine (10 mg), quetiapine (10 mg), olanzapine (5 mg), clozapine (100 mg), sulpiride (160 mg), metoclopramide (25 mg)) and peripheral(domperidone (10 mg)], L-NAME (5 mg) and L-arginine (100 mg) decreased the pressure within both sphincters. As a common effect, this decreased pressure was rescued, dose-dependently, by BPC 157 (10 μg, 10 ng) (also note that L-arginine and L-NAME given together antagonized each other's responses). With haloperidol, L-NAME worsened both the lower oesophageal and the pyloric sphincter pressure, while L-arginine ameliorated lower oesophageal sphincter but not pyloric sphincter pressure, and antagonized L-NAME effect. With domperidone, L-arginine originally had no effect, while L-NAME worsened pyloric sphincter pressure. This effect was opposed by L-arginine. All these effects were further reversed towards a stronger beneficial effect, close to normal pressure values, by the addition of BPC 157. In addition, NO level was determined in plasma, sphincters and brain tissue. Thiobarbituric acid reactive substances (TBARS) were also assessed. Haloperidol increased NO levels (in both sphincters, the plasma and brain), consistently producing increased TBARS levels in the plasma, sphincters and brain tissues. These effects were all counteracted by BPC 157 administration. In conclusion, we revealed that BPC 157 counteracts the anti-emetic neuroleptic class side effect of decreased pressure in sphincters and the dopamine/NO-system/BPC 157 relationship.

Keywords BPC 157 · Dopamine antagonists · Neuroleptics · NO-system · Rats

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Introduction

Dopamine antagonists (Bilic et al. 2001; Jelovac et al. 1999; Sikiric et al. 1985, 1986, 1987, 2000; Szabo and Neumeyer 1983) and nitric oxide synthase (NOS) blockade have ulcerogenic effects (Cesarec et al. 2013; Skorjanec et al. 2015). Thus, we challenged the anti-emetic issue of neuroleptics (Murray-Brown and Dorman 2015; Perkins and Dorman 2009). In rats, we studied how dopamine

neuroleptics/prokinetics interact with the lower oesophageal and pyloric sphincters, along with NO-agents, NOS-blocker, N(G)-nitro-L-arginine methyl ester (L-NAME), NOS-substrate, L-arginine and stable gastric pentadecapeptide BPC 157 (Sikiric et al. 2010, 2011, 2014, 2016, 2017).

This dopamine/NO-system blockade combination might be important because there is currently incomplete evidence from published randomized controlled trials to determine the effectiveness of haloperidol for nausea and vomiting in palliative care, which contrasts with haloperidol being commonly prescribed to relieve these symptoms (Murray-Brown and Dorman 2015; Perkins and Dorman 2009). Likewise, some studies have failed to demonstrate the effectiveness of metoclopramide and domperidone in GERD therapy (Grande et al. 1992) or their effects on sphincter pressure (Meng et al. 2016).

However, all these agents, in addition to dopamine receptors, may also affect various other receptors (e.g. metoclopramide is also a mixed 5-HT3 receptor antagonist/5-HT4 receptor agonist, and olanzapine has a higher affinity for 5-HT2A serotonin receptors than for D2 dopamine receptors).

Furthermore, in terms of the generally recognized significance of dopamine and the NO-system in the gastrointestinal tract (Paré and Glavin 1986; Sikiric et al. 2014; Szabo et al. 1987), the inhibition of either leads to stomach and/or duodenal ulcer induction or aggravation in rats (Bilic et al. 2001; Jelovac et al. 1999; Sikiric et al. 1985, 1986, 1987, 2000; Szabo and Neumeyer 1983; Cesarec et al. 2013; Skorjanec et al. 2015). However, the likely consequence of lower oesophageal and pyloric sphincter disability was not assumed and thereby not investigated or demonstrated. This dopamine/NO-system blockade combination should also be considered with regard to the higher doses commonly used in rat studies (Bilic et al. 2001; Jelovac et al. 1999; Sikiric et al. 1985, 1986, 1987, 2000; Szabo and Neumeyer 1983).

Indicative of the dopamine/NO relationship, NOS inhibitors induce catalepsy in mice, and an additive cataleptic effect occurs with haloperidol (Del Bel et al. 2005). Dopamine induced an increase in NOS activity in the renal medulla and cortex, whereas the hypotensive effect of L-arginine and hypertension induced by L-NAME were not modified by haloperidol (Costa et al. 2006). However, regarding this sphincter issue, a possible neuroleptics–prokinetics/NO-system relationship in vivo has not been studied.

To solve this dilemma, it may be helpful that pentadecapeptide BPC 157 interacts peripherally and centrally with both dopamine and the NO-system in various models and species, and also maintains gastrointestinal mucosa integrity and reinstates sphincter function (Sikiric et al. 2010, 2011, 2014, 2016, 2017). Interestingly, BPC 157 is an original anti-ulcer peptide in inflammatory bowel disease. In multiple sclerosis trials, LD-1 was not achieved and no side effects have been found in clinical trials (Klicek et al. 2013; Sikiric et al. 2010, 2011, 2014, 2016, 2017). In addition, BPC 157 is known to interact with several molecular pathways (Chang et al. 2011, 2014; Hsieh et al. 2017; Huang et al. 2015; Tkalcevic et al. 2007).

Thus, we hypothesized that a decrease in pressure within the lower oesophageal and pyloric sphincters is a class side effect in living rats administered with dopamine antagonists and/or NOS-blocker L-NAME, not reported heretofore. The regimens include those used as neuroleptics, typical and atypical, and those used as anti-emetics, acting centrally (haloperidol, fluphenazine, levomepromazine, chlorpromazine, quetiapine, olanzapine, sulpiride, clozapine, metoclopramide) and/or peripherally (domperidone) (Abi-Dargham 2014; Acosta and Camilleri 2015; Sikiric et al. 2014). Against this large background, we explored the potential of the stable gastric pentadecapeptide BPC 157 (Sikiric et al. 2010, 2011, 2014, 2016, 2017) and the NOS-substrate L-arginine. In particular, BPC 157 reinstates sphincter function that has failed due to acute and chronic oesophagitis, acute pancreatitis, oesophagocutaneous and duodenocutaneous fistulas or severe hyperkalaemia, and counteracts failed functioning of urethral and sphincter stress urinary incontinence (Barisic et al. 2013; Cesarec et al. 2013; Dobric et al. 2007; Jandric et al. 2013; Petrovic et al. 2006, 2011; Sikiric et al. 2010, 2011, 2014, 2016, 2017; Skorjanec et al. 2015) as well as pupil sphincter and counteracts atropine-mydriasis (Kokot et al. 2016), where its interaction with dopamine and NO-system may be a convincing background (Sikiric et al. 2011, 2014, 2016, 2017).

A basic extension of the previous findings on mucosal damaging effects of both dopamine and NO-system blockades (Bilic et al. 2001; Jelovac et al. 1999; Sikiric et al. 1985, 1986, 1987, 2000; Szabo and Neumeyer 1983; Cesarec et al. 2013; Skorjanec et al. 2015) on sphincter failure (Barisic et al. 2013; Cesarec et al. 2013; Dobric et al. 2007; Jandric et al. 2013; Petrovic et al. 2006, 2011; Sikiric et al. 2010, 2011, 2014, 2016, 2017) would prevail over previous studies that were not related to injurious conditions (Palheta et al. 2014; Braverman et al. 2011;Niedringhaus et al. 2008). In these studies, NO-lower oesophageal sphincter relaxation is based on the effect obtained in intact dogs (Palheta et al. 2014), rats (Braverman et al. 2011) and muscle strips (Niedringhaus et al. 2008). More specifically, our approach (Barisic et al. 2013; Cesarec et al. 2013; Dobric et al. 2007; Jandric et al. 2013; Petrovic et al. 2006, 2011; Sikiric et al. 2010, 2011, 2014, 2016, 2017) favours NO-related lower oesophageal and pyloric sphincter disability in addition to the neighbouring tissue damage; i.e. defects, oesophagitis

and even a systemic disturbance such as hyperkalaemia or arrhythmias (Barisic et al. 2013; Cesarec et al. 2013; Dobric et al. 2007; Jandric et al. 2013; Petrovic et al. 2006, 2011). As a novel combination, we would clearly suggest a possible NO-sphincter disability along with a neuroleptic/prokinetic effect. Of note, nitrergic inhibitory neurons of the myenteric plexus mediate inhibition in different parts of the stomach, including the pyloric sphincter (Ramkumar and Schulze 2005), while basal pyloric hypotension with normal nitrergic inhibition predisposes interstitial cells of Cajal (ICC)-deficient mice to duodenogastric bile reflux (Sivarao et al. 2008).

Thus, using rat lower oesophageal and pyloric sphincter failure assessments, as previously described (Barisic et al. 2013; Cesarec et al. 2013; Dobric et al. 2007; Petrovic et al. 2006, 2011; Skorjanec et al. 2015), we used a 1-week protocol to investigate whether dopamine antagonists acting centrally/peripherally and/or NOS-blocker L-NAME would induce a pressure decrease within sphincters and whether BPC 157 and/or L-arginine administration would benefit from the decreased pressure within the lower oesophageal and pyloric sphincters in rats. The experimental groups included (i) dopamine blockade (central and/or peripheral); (ii) NOS-blockade; and (iii) blockades combined, dopamine blockade and NOS-blockade (dopamine central blockade + NOS-blockade; dopamine peripheral blockade + NOS-blockade). Dopamine antagonists were given in the regimen known to produce gastric ulcer and/or central disturbances (Bilic et al. 2001; Jelovac et al. 1999; Sikiric et al. 1985, 1986, 1987, 2000). L-NAME was administered in the regimen known to aggravate gastric and oesophageal lesions and/or disabled sphincter functioning (Cesarec et al. 2013; Skorjanec et al. 2015). In addition, NO level was determined in plasma, sphincters and brain tissue. Accordingly, thiobarbituric acid reactive substance (TBARS)-oxidative stress was also assessed (Ohkawa et al. 1979).

Materials and methods

Animals

Wistar Albino male rats (200 g b.w.) were randomly assigned to the experiments (at least 10 animals per experimental group). The study was approved by the Local Ethics Committee at the School of Medicine (University of Zagreb, Zagreb, Croatia). Furthermore, all experiments were carried out under a blind protocol, and the effect was assessed by examiners who were completely unaware of the given protocol.

Drugs

Pentadecapeptide BPC 157 (GEPPPGKPADDAGLV, M.W.1419), (Diagen, Ljubljana, Slovenia) (10 μg/kg or 10 ng/kg) dissolved in saline was used in all experiments. The peptide, BPC 157, is part of the sequence of the human gastric juice protein BPC and is freely soluble in water at pH 7.0 and saline. It was prepared as previously described (Seiwerth et al. 2014; Sikiric et al. 2014; Tkalcevic et al. 2007), with 99% high-pressure liquid chromatography (HPLC) purity, expressing 1-des-Glypeptideasan impurity. Haloperidol, fluphenazine, levomepromazine, chlorpromazine, quetiapine, olanzapine, clozapine, sulpiride, metoclopramide, domperidone, L-NAME and L-arginine (Sigma, USA) were used accordingly (Sikiric et al. 2010, 2011, 2014, 2016, 2017).

Experimental protocol

All agents (/kg) were administered intraperitoneally once daily for 7 days, with the last application occurring 24 h before pressure assessment. Neuroleptics (typical, atypical) and prokinetics acting centrally [haloperidol (6.25, 16, 25 mg), fluphenazine (5 mg), levomepromazine (50 mg), chlorpromazine (10 mg), quetiapine (10 mg), olanzapine (5 mg), clozapine (100 mg), sulpiride (160 mg), metoclopramide (25 mg)] or peripherally [domperidone (10 mg)] were administered alone or together with BPC 157 (10 μg or 10 ng). L-NAME (5 mg) and L-arginine (100 mg) were administered alone and/or together, as well as with haloperidol (25 mg), domperidone (10 mg) or BPC 157 (10 μg). To ascertain the procedure assessment and the pressure values for lower oesophageal sphincter and for pyloric sphincter which were considered to be normal as determined previously, healthy rats received an equal volume (5 ml/kg) of saline intraperitoneally once daily for 7 days, with the last application at 24 h before pressure assessment.

Lower oesophageal sphincter pressure and pyloric sphincter pressure assessment

As described previously (Barisic et al. 2013; Cesarec et al. 2013; Dobric et al. 2007; Petrovic et al. 2006, 2011; Skorjanec et al. 2015), manometric evaluation (cm H2O) was performed in all rats, with a water manometer connected to the drainage port of the Foley catheter as described. The values of 68–76 cm H_2O for lower oesophageal sphincter and 68–74 cm H_2O for pyloric sphincter which were considered to be normal as determined previously were ascertained by saline (5 ml/kg intraperitoneally) given alone once daily for 7 days. The proximal side of the oesophageal incision, or the distal side of the duodenal incision, was ligated to prevent regurgitation (Barisic et al. 2013; Cesarec et al. 2013; Dobric et al. 2007; Petrovic et al. 2006, 2011; Skorjanec et al. 2015).

Nitric oxide determination in plasma, lower oesophageal and pyloric sphincter and brain tissue

We determined nitric oxide (NO) in plasma and tissue samples using the Griess reaction (Griess Reagent System, Promega, USA). Sulphanilamide was added to the homogenized tissue and incubated, after which *N*-1-naphthylethylenediamine dihydrochloride was added. The Griess reaction is based on the diazotization reaction in which acidified nitrite reacts with diazonium ions and, in a further step, is coupled to *N*-1-naphthylethylenediamine dihydrochloride, forming chromophoric azo derivate. Absorbance was measured at 540 nm, using sodium nitrite solution as standard. NO levels are reported in μmol/mg protein. Proteins are determined using a commercial kit (BioRad Protein DR Assay Reagent Kit, USA).

TBARS determination in plasma, lower oesophageal and pyloric sphincter and brain tissue

Measurement of TBARS concentration with thiobarbituric acid following the formation of TBARS was performed on tissue samples (Ohkawa et al. 1979). Briefly, tissue homogenate was mixed with a solution containing trichloroacetic acid and incubated to precipitate the proteins. Following centrifugation, thiobarbituric acid was added. The mixture was heated, and the absorbance of the supernatant fraction was determined (532 nm). A standard curve was prepared with serial dilutions of standard 1,1,3,3-tetramethoxypropane. The results are expressed as μmol/mg of protein (determined with bovine serum albumin as standard using BioRad Protein Reagent Kit).

Statistical analysis

Statistical analysis was performed using a parametric two-way mixed model ANOVA (one factor is repeated measures) and Student–Newman–Keuls test to compare the differences between groups. Additionally, a non-parametric Kruskal–Wallis and a post hoc Mann–Whitney *U* test were used. Fisher's exact probability test was used to assess the differences in frequencies between groups. *P* values of 0.05 or less were considered to be statistically significant.

Results

Regarding this sphincter issue and the evidence that a possible neuroleptics–prokinetics/NO-system relationship in vivo has not been studied, we used an additional procedure for ascertaining. The ascertaining protocol (saline intraperitoneally once daily for 7 days in healthy rats) revealed that the values within the values for lower oesophageal sphincter and pyloric sphincter which were considered to be normal as determined before.

Effects of haloperidol, fluphenazine, levomepromazine, chlorpromazine, quetiapine, olanzapine, sulpiride, clozapine, metoclopramide and domperidone

Considering the previously determined normal values in normal rats in the lower oesophageal and pyloric sphincters and, specifically, the values obtained with saline protocol given intraperitoneally in healthy rats once daily for 7 days, all the applied agents, neuroleptics (typical and atypical) and prokinetics (acting centrally and/or peripherally) induced a decrease in pressure within both the lower oesophageal and the pyloric sphincters (Figs. 1, 2, 3, 4, 5), an effect which

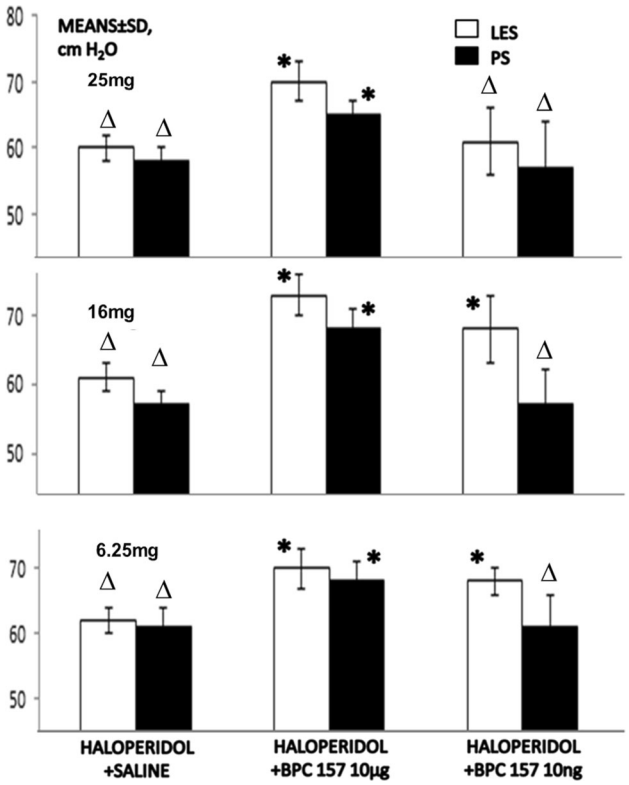

Fig. 1 Pressure within sphincters after administration of haloperidol alone or with BPC 157. Pressure within lower oesophageal sphincter (LES) (*empty bars*) and pyloric sphincter (PS) (*black bars*). Pressure outcome after administration of haloperidol (6.25 mg/kg, 16 mg/kg, 25 mg/kg) and saline (5 ml/kg) or BPC 157 (10 μg/kg, 10 ng/kg) intraperitoneally, once daily through 7 days, with the last application 24 h before pressure assessment. Saline (5 ml/kg) administered intraperitoneally once daily for 7 days in healthy rats, with the last application 24 h before pressure assessment, revealed the values (cm H_2O) of 72 ± 2 for lower esophageal sphincter and 70 ± 3 for the pyloric sphincter. The values were within the values which were considered to be normal as determined before. *$P < 0.05$ at least, vs. haloperidol + saline; Δ$P < 0.05$ at least, vs. saline in healthy

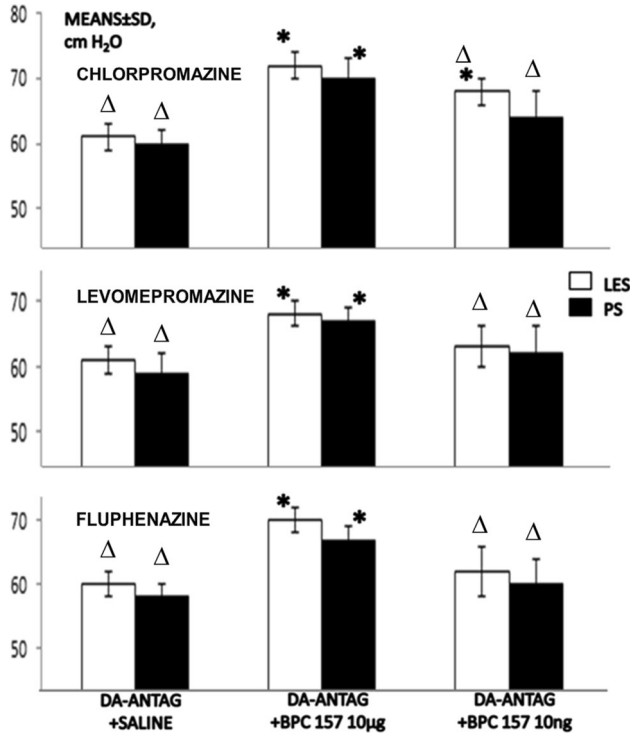

Fig. 2 Pressure within sphincters after administration of chlorpromazine, levomepromazine and fluphenazine alone or with BPC 157. Pressure within the lower oesophageal sphincter (LES) (*empty bars*) and pyloric sphincter (PS) (*black bars*). Pressure outcome after administration of dopamine antagonist (DA-ANTAG), chlorpromazine (10 mg/kg), levomepromazine(50 mg/kg), fluphenazine (5 mg/kg) and saline (5 ml/kg) or BPC 157 (10 µg/kg, 10 ng/kg) intraperitoneally, once daily through 7 days, with the last application 24 h before pressure assessment. Saline (5 ml/kg) administered intraperitoneally once daily for 7 days in healthy rats, with the last application 24 h before pressure assessment, revealed the values (cm H_2O) of 72 ± 2 for lower oesophageal sphincter and 70 ± 3 for pyloric sphincter. The values were within the values which were considered to be normal as determined before. *$P < 0.05$ at least, vs. dopamine antagonist + saline; Δ$P < 0.05$ at least, vs. saline in healthy

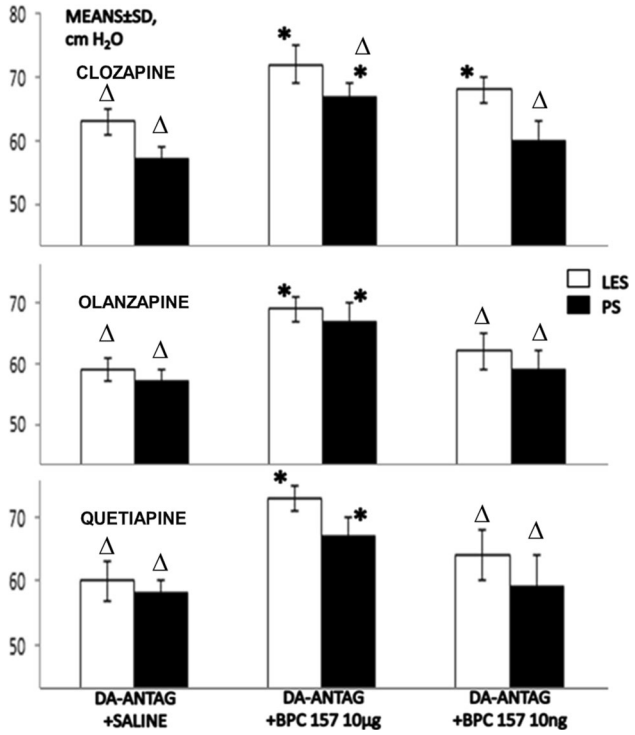

Fig. 3 Pressure within sphincters after administration of clozapine, olanzapine, quetiapine alone or with BPC 157. Pressure within lower oesophageal sphincter (LES) (*empty bars*) and pyloric sphincter (PS) (*black bars*). Pressure outcome after administration of dopamine antagonist (DA-ANTAG), clozapine (100 mg/kg), olanzapine (5 mg/kg), quetiapine (10 mg/kg) and saline (5 ml/kg) or BPC 157 (10 µg/kg, 10 ng/kg) intraperitoneally, once daily through 7 days, with the last application 24 h before pressure assessment. Saline administered (5 ml/kg) intraperitoneally once daily for 7 days in healthy rats, with the last application 24 h before pressure assessment, revealed the values (cm H_2O) of 72 ± 2 for lower oesophageal sphincter and 70 ± 3 for pyloric sphincter. The values were within the values which were considered to be normal as determined before. *$P < 0.05$ at least, vs. dopamine antagonist + saline; Δ$P < 0.05$ at least, vs. saline in healthy

was not dose related, at least with haloperidol. The pylorus sphincter seemed to be more affected.

Effect of BPC 157 on the effect of haloperidol, fluphenazine, levomepromazine, chlorpromazine, quetiapine, olanzapine, sulpiride, clozapine, metoclopramide and domperidone

The counteracting effect of BPC 157 on pressure decreases in the sphincters rescued the decreased pressure induced by all neuroleptics, metoclopramide and domperidone in both the lower oesophageal and the pyloric sphincters. This may be seen with µg regimens (Fig. s1, 2, 3, 4, 5). The BPC 157 effect included all the increasing doses of haloperidol (Fig. 1).

Effect of L-NAME and L-arginine

Considering the previously determined normal values in normal rats in the lower oesophageal and the pyloric sphincters, given as individual agents, both L-arginine (L-arginine-rats) and L-NAME (L-NAME-rats) produced a decrease in both lower oesophageal and pyloric sphincter pressures, which were probably distinct and NO-specific effects (L-arginine produced a small decrease, while L-NAME induced a more prominent decrease) because they attenuated each other's responses when combined (L-NAME + L-arginine-rats) (Fig. 5).

Effect of BPC 157 on the effect of L-NAME and on the effect of L-arginine

Decreased values in rats administered with L-arginine (L-arginine-rats) and more greatly decreased values in rats

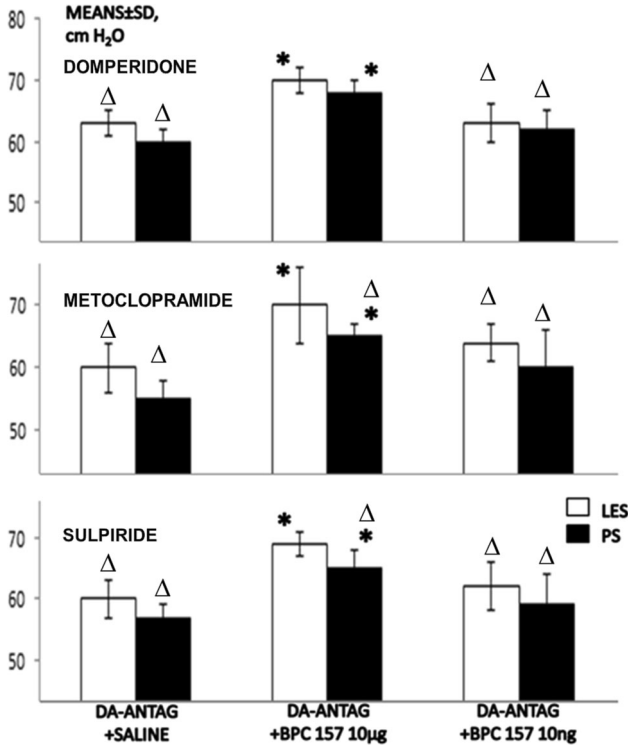

Fig. 4 Pressure within sphincters after administration of domperidone, metoclopramide and sulpiride alone or with BPC 157. Pressure within lower oesophageal sphincter (LES) (*empty bars*) and pyloric sphincter (PS) (*black bars*). Pressure outcome after administration of dopamine antagonist (DA-ANTAG), domperidone (10 mg/kg), metoclopramide (25 mg/kg) and sulpiride (160 mg/kg) and saline (5 ml/kg) or BPC 157 (10 µg/kg, 10 ng/kg) intraperitoneally, once daily through 7 days, with the last application 24 h before pressure assessment. Saline (5 ml/kg) administered intraperitoneally once daily for 7 days in healthy rats, with the last application 24 h before pressure assessment, revealed the values (cm H_2O) of 72 ± 2 for lower oesophageal sphincter and 70 ± 3 for pyloric sphincter. The values were within the values which were considered to be normal as determined before. $^*P < 0.05$ at least, vs. dopamine antagonist + saline; $\Delta P < 0.05$ at least, vs. saline in healthy

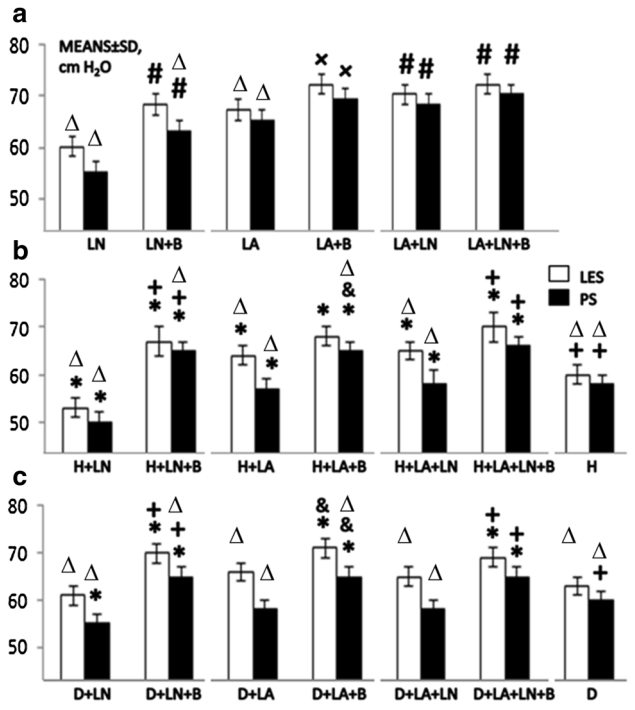

Fig. 5 Pressure within sphincters after administration of haloperidol, domperidone, L-NAME, L-arginine and BPC 157. Pressure within lower oesophageal sphincter (LES) (*empty bars*) and pyloric sphincter (PS) (*black bars*). **a** Pressure outcome after administration of L-NAME (LN), L-arginine (LA) and/or BPC 157 (**b**), $\#P < 0.05$ at least, vs. L-NAME; $xP < 0.05$ at least, vs. L-arginine. **b** Pressure outcome after administration of dopamine antagonist [haloperidol (H)] acting mostly centrally with NOS-blockade (L-NAME), NOS-substrate (L-arginine) and BPC 157. $^*P < 0.05$ at least, vs. haloperidol, $+ P < 0.05$ at least, vs. haloperidol + L-NAME, $\&P < 0.05$ at least, vs. haloperidol + L-arginine. **c** Pressure outcome after administration of dopamine antagonist [domperidone (D)] acting mostly peripherally with NOS-blockade (L-NAME), NOS-substrate (L-arginine) and BPC 157. Saline (5 ml/kg) administered intraperitoneally once daily for 7 days in healthy rats, with the last application 24 h before pressure assessment, revealed the values (cm H_2O) of 72 ± 2 for lower oesophageal sphincter and 70 ± 3 for pyloric sphincter. The values were within the values which were considered to be normal as determined before. $^*P < 0.05$ at least, vs. domperidone, $+P < 0.05$ at least, vs. domperidone +L-NAME, $\&P < 0.05$ at least, vs. domperidone +L-arginine; $\Delta P < 0.05$ at least, vs. saline in healthy

administered with L-NAME (L-NAME-rats) were increased with the addition of BPC 157, but not over normal values (L-arginine + BPC 157-rats; L-NAME + BPC 157-rats; L-NAME + L-arginine + BPC 157-rats) (Fig. 5).

Haloperidol and L-NAME and L-arginine

In rats administered haloperidol, L-NAME led to an additional decrease in pressure for both the lower oesophageal and the pyloric sphincters. L-arginine rescued only the lower oesophageal sphincter. In rats administered haloperidol and L-NAME (haloperidol + L-NAME-rats), L-arginine rescued both the lower oesophageal and pyloric sphincters (haloperidol + L-NAME + L-arginine-rats) (Fig. 5).

BPC 157

A consistent increase in both lower oesophageal and pyloric sphincter pressure resulted from the addition of BPC 157 to rats administered haloperidol and L-NAME (haloperidol + L-NAME-rats) or L-arginine (haloperidol + L-arginine-rats) and L-NAME and L-arginine (haloperidol + L-NAME + L-arginine-rats). This BPC 157 treatment brought the pressure values in those rats close to normal values (haloperidol + L-NAME + BPC 157-rats; haloperidol + L-arginine + BPC 157 rats haloperidol + L-NAME + L-arginine + BPC 157-rats) (Fig. 5).

Domperidone and L-NAME and L-arginine

In rats administered domperidone presenting with already decreased pressure values in both sphincters, L-NAME additionally decreased values in the pyloric sphincter, and L-arginine showed no rescuing effects on its own. However, in rats administered with domperidone and L-NAME, L-arginine administration reversed the pylorus pressure (domperidone + L-NAME + L-arginine-rats) to the values found in rats administered only domperidone (Fig. 5).

BPC 157

The addition of BPC 157 was consistently effective in increasing the pressure values. Rats administered domperidone and L-NAME (domperidone + L-NAME-rats) or L-arginine (domperidone + L-arginine-rats) and L-NAME and L-arginine (domperidone + L-NAME + L-arginine-rats) exhibited a consistent increase in both lower oesophageal and pyloric sphincter pressure with the addition of BPC 157. The pressure values in those rats were close to normal values (haloperidol + L-NAME + BPC 157-rats; haloperidol + L-arginine + BPC 157-rats haloperidol + L-NAME + L-arginine + BPC 157-rats) (Fig. 5).

In summary, we noted decreases in lower oesophageal and pyloric sphincter pressure as a common effect of all neuroleptics, metoclopramide, domperidone and rescue, which was dose dependent with BPC 157. Interestingly, while a decrease in pressure appeared in the lower oesophageal and pyloric sphincter pressure with L-arginine and L-NAME, both were rescued with BPC 157.

However, L-NAME augmented the effects of haloperidol on the lower oesophageal and the pyloric sphincters, as well as the effect of domperidone on the pyloric sphincter. L-arginine rescued the lower oesophageal sphincter when haloperidol was administered, but not domperidone, and it interfered with the effect of L-NAME in both haloperidol and domperidone rats. All of these rats exhibited a consistent increase towards normal values in both lower oesophageal and pyloric sphincter pressures with the addition of BPC 157.

NO levels in plasma, lower oesophageal and pyloric sphincter and brain tissue

Haloperidol consistently increased NO values (lower oesophageal and pyloric sphincter, plasma and brain). All these increased values were counteracted by the addition of BPC 157 (Fig. 6).

Levels of TBARS in plasma, lower oesophageal and pyloric sphincter and brain tissue

Haloperidol consistently increased TBARS values (lower oesophageal and pyloric sphincter, plasma and brain). All these increased values were counteracted by the addition of BPC 157 (Fig. 7).

Discussion

We emphasized various unresolved issues of anti-emetic, neuroleptic applications in both patients and experimental studies (Bilic et al. 2001; Jelovac et al. 1999; Murray-Brown and Dorman 2015; Perkins and Dorman 2009; Grande et al. 1992; Meng et al. 2016; Sikiric et al. 1985, 1986, 1987, 2000; Szabo and Neumeyer 1983). Controlled trials object to the common prescription of haloperidol (Murray-Brown and Dorman 2015; Perkins and Dorman 2009). The ulcerogenic potential of dopamine antagonists (Bilic et al. 2001; Jelovac et al. 1999; Sikiric et al. 1985, 1986, 1987, 2000; Szabo and Neumeyer 1983) was not related to possible sphincter failure, and relationships with the NO-system were not defined [NOS blockade also has ulcerogenic effects (Cesarec et al. 2013; Skorjanec et al. 2015)]. Thus, we studied how dopamine neuroleptics/prokinetics interact with the lower oesophageal and pyloric sphincters along with NO-agents, NOS-blocker, L-NAME, NOS-substrate, L-arginine and stable gastric pentadecapeptide BPC 157 in rats (Sikiric et al. 2010, 2011, 2014, 2016, 2017).

We found the characteristic effect of a common pressure decrease in both sphincters alongside all dopamine antagonists, a probable class effect that was counteracted by BPC 157 administration. Furthermore, the additional involvement of different receptors in the effects of these agents, as mentioned earlier (i.e. metoclopramide vs. olanzapine; haloperidol vs. clozapine vs. quetiapine, etc.), would additionally emphasize the significance of the uniform counteraction obtained by BPC 157 administration.

Most importantly for dopamine/NO-system/BPC 157 relationships in normal and disturbed conditions, NO-agents, L-NAME and L-arginine are distinctive under healthy conditions and in the presentation of dopamine central and peripheral blockade in the lower oesophageal and pyloric sphincters. Given as individual agents in healthy rats, all neuroleptics, metoclopramide, domperidone, L-arginine and L-NAME decreased the pressures within the lower oesophageal and the pyloric sphincters. As a common effect, the decreased pressure was rescued dose-dependently with BPC 157 (also note that L-arginine and L-

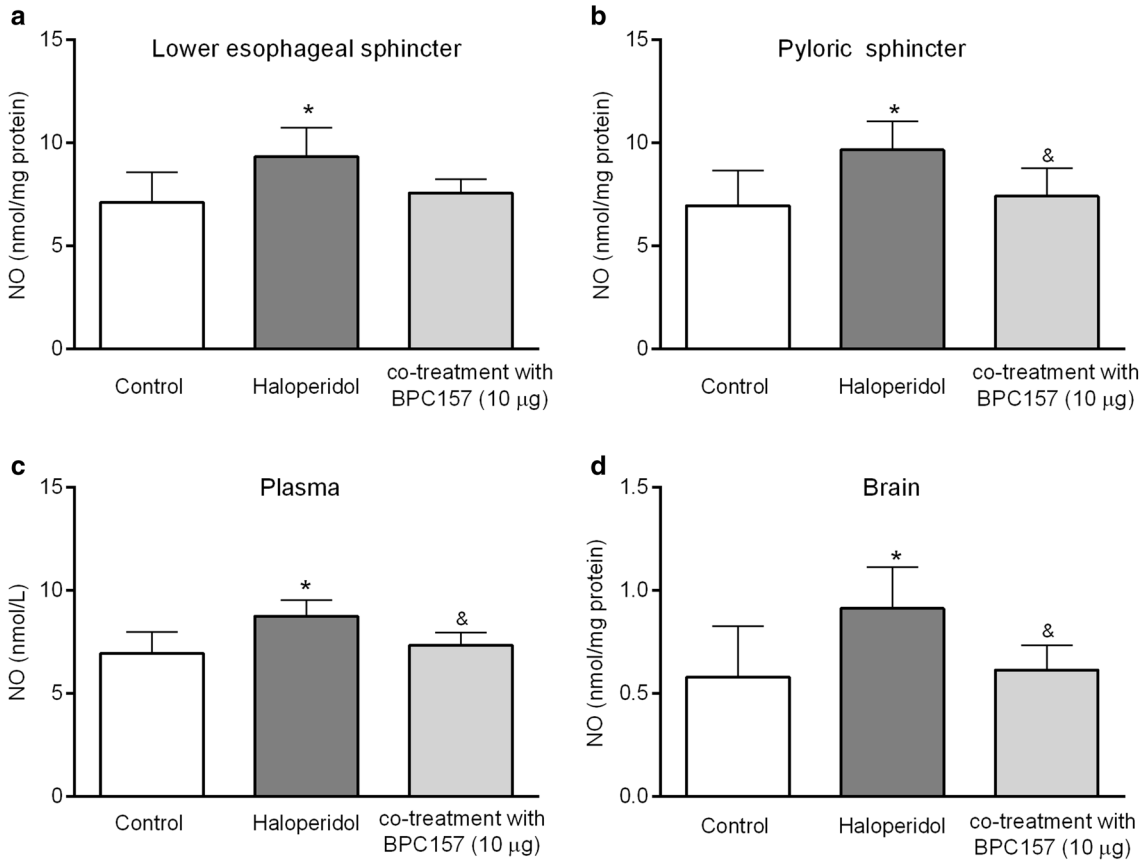

Fig. 6 Nitric oxide levels in different tissues. Nitric oxide levels in lower oesophageal (**a**) and pyloric (**b**) sphincter, plasma (**c**) and brain (**d**) of control, haloperidol- and haloperidol + BPC 157 (10 μg)-treated rats. Using colorimetric assay, we determined levels of NO. *$P < 0.05$ versus control group and &$P < 0.05$ versus haloperidol-treated group

NAME administered together antagonized each other's responses). Administered with dopamine blockade, and with haloperidol, L-NAME worsened both lower oesophageal sphincter pressure and pyloric sphincter pressure, while L-arginine ameliorated (lower oesophageal sphincter pressure) and antagonized the effect of L-NAME (lower oesophageal sphincter and pyloric sphincter pressure).With domperidone, L-arginine originally had no effect, while L-NAME worsened pyloric sphincter pressure. This effect was opposed by L-arginine. As mentioned above, all these effects were further reversed towards a stronger beneficial effect, close to normal pressure values, with the addition of BPC 157. In principle, the effects of L-NAME/L-arginine/BPC 157 are in line with the results obtained in other sphincter failure models (Barisic et al. 2013; Cesarec et al. 2013; Skorjanec et al. 2015; Kokot et al. 2016).

This means that, as individual agents, both L-NAME and L-arginine affect sphincter pressure in particular ways, in which each of the effects is specific (i.e. NO-related) as a particular aspect of the NO-system dual (L-NAME vs. L-arginine) role (vs. combination) (for reviews see Moncada et al. 1991; Whittle et al., 1992) and are prepared in relation to the dopamine system in the particular case of the pressure within the lower oesophageal and pyloric sphincters. Namely, given together (L-NAME + L-arginine), they regularly attenuated or antagonized each other's response. Likewise, the remaining pathology (decreased sphincter pressure) in the L-NAME + L-arginine animals means that other system(s) (i.e. dopamine, central and/or peripheral, BPC 157-system) may function along with the NO-system (previously supposed to be immobilized by the mutual actions of the combination of L-NAME and L-arginine). Thus, with central and peripheral dopamine blockade, L-NAME exhibited worsening effects, and with central dopamine blockade, L-arginine exhibited amelioration (note that, in domperidone rats, L-arginine antagonized the effect of L-NAME but not the effect of domperidone itself).

In practice, for L-NAME/L-arginine, this means two pharmacologically distinct mechanisms with opposite effects on the same signalling NO-pathway confronted with regularly unresolvable failure (dopamine central and peripheral blockade leading to sphincter failure). Additionally, in practice, this means two distinct end points that

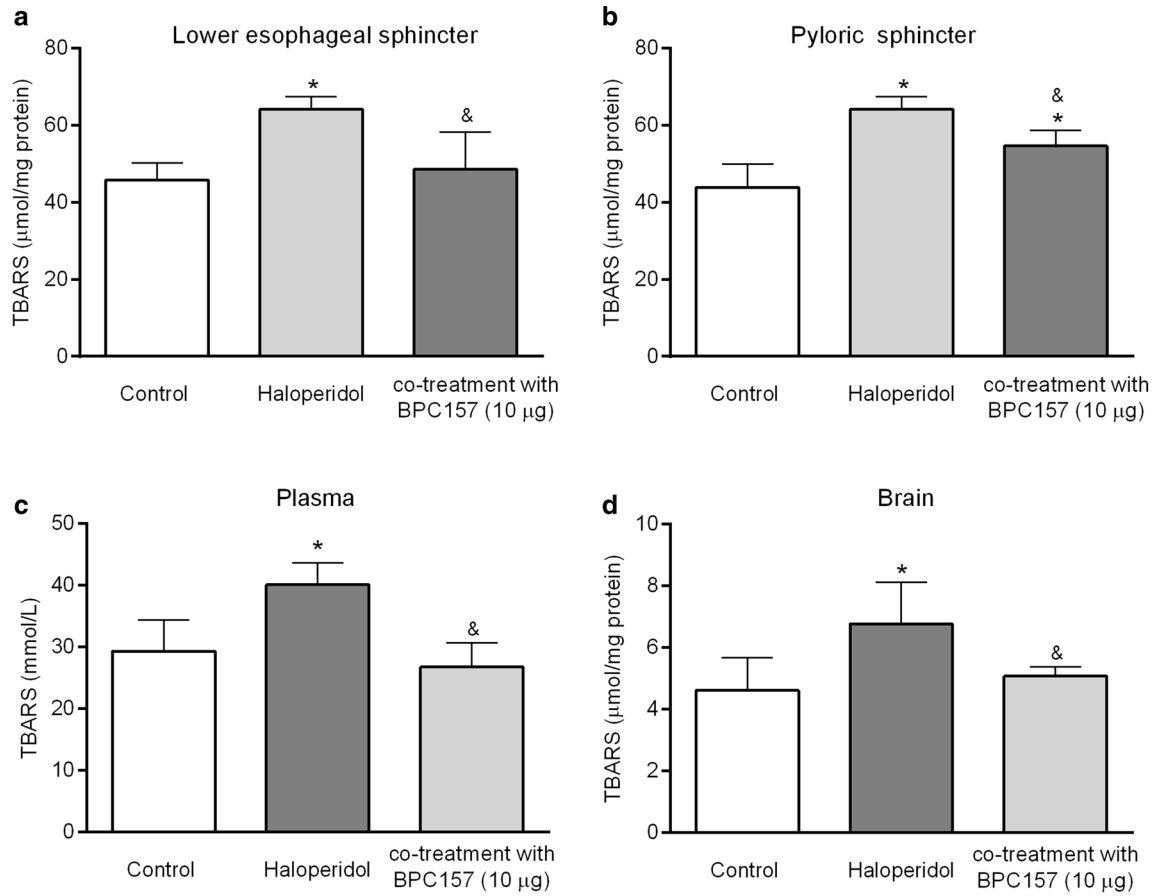

Fig. 7 TBARS levels in different tissues. TBARS levels in lower oesophageal (**a**) and pyloric (**b**) sphicter, plasma (**c**) and brain (**d**) of control, haloperidol- and haloperidol + BPC 157 (10 μg)-treated rats. Using colorimetric assay, we determined the levels of TBARS as a measure of lipid peroxidation in the tissue. $*P < 0.05$ versus control group and $\&P < 0.05$ versus haloperidol-treated group

should both be addressed to achieve the presentation seen in rats administered with BPC 157. Note that, to support the regulation of either of the effects of L-NAME and L-arginine, BPC 157 instantly prevents and reverses L-NAME-induced hypertension, as well as L-arginine-induced hypotension (Sikiric et al. 1997). Likewise, owing to its counteraction of prolonged bleeding and thrombocytopaenia, after amputation and/or anticoagulant application, BPC 157 counteracts both L-NAME (thrombocytopaenia) and L-arginine (prolonged bleeding, thrombocytopaenia) side effects (Stupnisek et al. 2015). Additionally, in a pupil assay, a common L-NAME/L-arginine point was accordingly noted (miosis, L-NAME/L-arginine antagonized each other's responses) and explained in terms of an interaction with the cholinergic system (Kokot et al. 2016), while BPC 157 counteracts both L-NAME (miosis) and L-arginine (miosis), and, interestingly, BPC 157 also counteracts atropine mydriasis (Kokot et al. 2016).

In practice, for the dopamine/NO-system, this indicates two pharmacologically distinct systems and mechanisms with maintaining effects on the same signalling target (i.e. sphincter) confronted with the same regularly unresolvable, but exaggerated failure (both dopamine and NO-system central and peripheral blockade leading to sphincter failure). Namely, as demonstrated, a further decrease when combined (domperidone and L-NAME or haloperidol and L-NAME) implies that neither of the effects of peripheral or central dopamine blockade or NOS-blockade is maximal, that they could be mutually potentiated and that they are likely to collaborate in the maintenance of the normal function of both the lower oesophageal and the pyloric sphincters.

Therefore, these results should be taken into account with the consistently increased NO levels (lower oesophageal and pyloric sphincter, plasma and brain) by haloperidol. In theory, this may be seen as NO-system compensation for dopamine blockade (and, thereby, is likely to be opposed by L-NAME). As an alternative theory in haloperidol rats, this leads to NO excessive release generated by the inducible isozyme (Luetic et al. 2017). As noted recently in our cyclophosphamide studies (Luetic et al. 2017), this damages the vascular wall and other tissue

cells, especially in combination with reactive oxygen intermediates [·NO is likely to rapidly react with $\cdot O_2$ to form peroxynitrite, which may be directly cytotoxic (Oldham and Bowen 1998)] while failing in endothelial production and resulting in further aggravation by L-NAME, which was inhibited by L-arginine. In addition, TBARS values were also increased, indicating increased lipid peroxidation in plasma, sphincters and brain tissues. Thus, it seems to be a common peripheral and central phenomenon that is consistently produced. These effects were all counteracted by BPC 157 administration. Interestingly, as a possible antioxidant, the pentadecapeptide BPC 157 contains four carboxylic groups, which could all be active in scavenger processes. If reactivated (e.g. by glutathione or enzymatic), the overall activity could be very high. BPC 157 also counteracted other free-radical-induced lesions (Ilic et al. 2010; Luetic et al. 2017; Sikiric et al. 1993).

Illustratively, in an ability to rescue failed sphincters, BPC 157 in normal rats increases lower oesophageal sphincter pressure, but decreases pyloric sphincter pressure (Dobric et al. 2007; Petrovic et al. 2006), thus indicating an anti-reflux effect. Additionally, unlike injurious conditions, it does not affect normal pupil (Kokot et al. 2016) or urethral sphincters (Jandric et al. 2013), thus exhibiting a sphincter-specific and injury-specific effect (Sikiric et al. 2014). The particular success of BPC 157 sphincter rescue therapy includes lower oesophageal and pyloric sphincters when disabled by various procedures [i.e. stretching sphincters with temporal tube insertion (Dobric et al. 2007; Petrovic et al. 2006, 2011), potassium chloride overdose application (Barisic et al. 2013), bile duct ligation-induced pancreatitis (Petrovic et al. 2011) and oesophagocutaneous (Cesarec et al. 2013) and duodenocutaneous fistula creation (Skorjanec et al. 2015)]. Thus, sphincter failure as part of a syndrome could be rescued alongside the rescue of the complete syndrome (Barisic et al. 2013; Cesarec et al. 2013; Dobric et al. 2007; Petrovic et al. 2006, 2011; Skorjanec et al. 2015) [i.e. potassium chloride overdose application also induced hyperkalaemia, hypertension, gastric lesions, arrhythmia and short-term fatal outcomes, aggravated by L-NAME, and all were completely counteracted by BPC 157 administration (Barisic et al. 2013)]. Accordingly, in various models and species (Balenovic et al. 2009, 2012; Barisic et al. 2013; Boban-Blagaic et al. 2006; Cesarec et al. 2013; Grabarevic et al. 1997; Klicek et al. 2008; Sikiric et al. 1997, 2011, 2014; Skorjanec et al. 2015; Stupnisek et al. 2015; Zemba et al. 2015), BPC 157 counteracted L-NAME effects better than L-arginine and induced NO-release in gastric mucosa from rat stomach tissue homogenates, even in conditions in which L-arginine was not working (Sikiric et al. 1997; Turkovic et al. 2004).

In conclusion, the importance of BPC 157 and particular counteractions include attenuated gastrointestinal lesions induced by a peripherally/centrally disabled dopamine system (Bedekovic et al. 2003; Bilic et al. 2001; Petrovic et al. 2006; Sikiric et al. 1999, 2001a, b, 2011), dopamine release overstimulation (Jelovac et al. 1998; Sikiric et al. 2002), dopamine receptor blockade (Jelovac et al. 1999; Sikiric et al. 2000) and dopamine neuron destruction (Sikiric et al. 1999) alongside counteracted akinesia or catalepsy (Jelovac et al. 1999; Sikiric et al. 2000). It also implies that, when BPC 157 is given peripherally, acutely and chronically, the release of the brain's serotonin (substantia nigra) (Tohyama et al. 2004) and resulting peptides act either centrally or indirectly by binding sites in the gut at some visceral receptive relay of the central nervous system (Aziz and Thompson 1998; Tohyama et al. 2004; Sikiric et al. 2016).

Thus, sphincter failure with dopamine antagonists and/or L-NAME could be a hallmark of ongoing injury (Bedekovic et al. 2003; Jelovac et al. 1998; Petrovic et al. 2006; Sikiric et al. 1999, 2001, 2002) alongside the injurious effects of dopamine antagonists or L-NAME itself (Balenovic et al. 2009, 2012; Barisic et al. 2013; Boban-Blagaic et al. 2006; Grabarevic et al. 1997; Klicek et al. 2008; Sikiric et al. 1997; Stupnisek et al. 2015, Zemba et al. 2015). Similarly, sphincter failure that can be reversed may further implicate the importance of BPC 157 in the corresponding therapy. A further argument is the disability of both sphincters, with a large number of agents acting both centrally (haloperidol, fluphenazine, levomepromazine, chlorpromazine, quetiapine, olanzapine, clozapine and sulpiride) and peripherally (domperidone) (Abi-Dargham 2014; Acosta and Camilleri 2015). This is in accordance with evidence that the induction or aggravation of experimental gastric (Sikiric et al. 1986) or duodenal ulcers (Gallagher et al. 1987) by dopamine antagonists or the therapeutic effects of dopamine agonists (Sikiric et al. 1991) probably involve a peripheral component. In addition to the suggested common dopamine component, the particular activities of the given agent and the distinction between the receptors involved (quetiapine less affects dopamine receptors (Mauri et al. 2014)) might indicate that each of the combinations might be responsible for prolonged sphincter failure.

Finally, this study raised several issues that will require further studies to elucidate. For instance, it is possible that the described effects that cause sphincter failure are different from the effects that may be seen from nausea and vomiting in palliative care, which are commonly ascribed to effects on the vomiting centre, chemoreceptor trigger zone, cerebral cortex and vestibular system as the four main sites of activity (Glare et al. 2011). Similarly, the negative effects on sphincters could probably be overridden by the effects on nausea and vomiting due to effects on the vomiting centre, chemoreceptor trigger zone, cerebral cortex and vestibular system (Glare et al. 2011).

In conclusion, we revealed that BPC 157 counteracts the anti-emetic neuroleptic class side effect of decreased pressure in sphincters and the dopamine/NO-system/BPC 157 relationship.

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