ELSEVIER

European Journal of Pharmacology 332 (1997) 23–33

ejp

The influence of a novel pentadecapeptide, BPC 157, on N^G-nitro-L-arginine methylester and L-arginine effects on stomach mucosa integrity and blood pressure

Predrag Sikirić *, Sven Seiwerth, Željko Grabarević, Rudolf Ručman, Marijan Petek, Vjekoslav Jagić, Branko Turković, Ivo Rotkvić, Stjepan Miše, Ivan Zoričić, Paško Konjevoda, Darko Perović, Ljubica Jurina, Jadranka Šeparović, Miro Hanževački, Branka Artuković, Mirna Bratulić, Marina Tišljar, Miro Gjurašin, Pavao Miklić, Dinko Stančić-Rokotov, Zoran Slobodnjak, Nikola Jelovac, Anton Marović

Center for Digestive Diseases, Medical Faculty, Medical and Veterinary Faculty, University of Zagreb, P.O. Box 916, Šalata 11, 10000 Zagreb, Croatia

Received 24 February 1997; revised 20 May 1997; accepted 23 May 1997

Abstract

The known effects of a novel stomach pentadecapeptide BPC157 (10 μg or 10 ng/kg), namely its salutary activity against ethanol (96%, i.g.)-induced gastric lesions (simultaneously applied i.p.) and in blood pressure maintenance (given i.v.), were investigated in rats challenged with a combination of N^G-nitro-L-arginine methylester (L-NAME) (5 mg/kg i.v.), a competitive inhibitor of endothelium nitric oxide (NO)-generation and NO precursor, L-arginine (200 mg/kg i.v.) (D-arginine was ineffective). In the gastric lesions assay, NO agents were given 5 min before ethanol injury and BPC 157 medication. Given alone, BPC157 had an antiulcer effect, as did L-arginine, but L-NAME had no effect. L-NAME completely abolished the effect of L-arginine, whereas it only attenuated the effect of BPC 157. After application of the combination of L-NAME + L-arginine, the BPC157 effect was additionally impaired. In blood pressure studies, compared with L-arginine, pentadecapeptide BPC 157 (without effect on basal normal values) had both a mimicking effect (impaired L-NAME-blood pressure increase, when applied prophylactically and decreased already raised L-NAME values, given at the time of the maximal L-NAME-blood pressure increase (i.e., 10 min after L-NAME)) and preventive activity (L-arginine-induced moderate blood pressure decrease was prevented by BPC 157 pretreatment). When BPC 157 was given 10 min after L-NAME + L-arginine combination, which still led to a blood pressure increase, its previously clear effect (noted in L-NAME treated rats) disappeared. In vitro, in gastric mucosa from rat stomach tissue homogenates, BPC 157, given in the same dose (100 μM) as L-arginine, induced a comparable generation of NO. But, BPC 157 effect could not be inhibited by L-NAME, even when L-NAME was given in a tenfold (100 versus 1000 μM) higher dose than that needed for inhibition of the L-arginine effect. NO synthesis was blunted when the pentadecapeptide BPC 157 and L-arginine were combined. In summary, BPC 157 could interfere with the effects of NO on both gastric mucosal integrity and blood pressure maintenance in a specific way, especially with L-arginine, having a more prominent and/or particularly different effect from that of NO. © 1997 Elsevier Science B.V.

Keywords: Pentadecapeptide BPC 157; Peptide BPC; Nitric oxide (NO); Gastrointestinal mucosal integrity; Blood pressure maintenance; Gastric lesion; Hypotension; N^G-Nitro-L-arginine methylester; L-Arginine; Stomach mucosa

1. Introduction

The possibility that a novel stomach pentadecapeptide BPC 157, (thought to be essential for beneficial activity of an organoprotective protein isolated from human gastric

* Corresponding author. Tel.: (385-1) 456-6833/456-6834/456-6836; Fax: (385-1) 424-001/275-382.

PII S0014-2999(97)01033-9

juice (named BPC)) and which has noted beneficial effects (Sikiric et al., 1993b, 1994, 1996a,b, 1997a,b; Seiwerth et al., 1997; Grabarevic et al., 1997) could interact with NO was investigated.

Based on its salutary effect on ethanol-induced lesions and on other lesions gastrointestinal lesions induced by various challengers (i.e., prolonged stress, nonsteroidal anti-inflammatory analgetics, cystamine, trinitrobenzene sulfonic acid, dinitrofluorbenzene sulfonic acid, acute pancreatitis, somatosensory neurons depletion) (Sikiric et al., 1993b, 1994, 1996a,b, 1997a,b; Veljaca et al., 1994a,b, 1995b, Paré and Klucyznski, 1994; Sandor et al., 1996) BPC was recently claimed to have a 'cytoprotective' effect, particularly on the gastrointestinal intestinal mucosa, along with a particular beneficial effect on endothelium integrity (Sikiric et al., 1993b, 1994). Considering the existence of BPC-157 in gastric juice and its unusual stability (Sikiric et al., 1993b) (i.e., incubated in human gastric juice or in water, this pentadecapeptide is not subjected to degradation at least for 24 h (Veljaca et al., 1995a)) it is likely that this peptide has some biological actions. It is suggested that BPC, which is formed constitutively in the gastric mucosa (body) and which is present in gastric juice (Sikiric et al., 1993a,b,c, 1994, 1996a,b, 1997a,b,c), protect the stomach against injury. Evidence shows that this pentadecapeptide has a strong anti-inflammatory activity in both acute and chronic inflammation models (Sikiric et al., 1993a, 1997c), reduces the release of inflammatory mediators (i.e., myeloperoxidase, leukotriene B_4, tromboxane B_2) in vitro and in vivo (Veljaca et al., 1994a,b, 1995b), markedly attenuates various experimental lesions in other organs (i.e., liver (Sikiric et al., 1993c), pancreas (Sikiric et al., 1996b), heart (Sikiric et al., 1993b; Bosnjak et al., 1994; Grabarevic et al., 1997)) and promotes wound and fracture healing in rats (Sikiric et al., 1993b, Seiwerth et al., 1997). Interestingly, an effect on disturbed blood pressure (e.g., Goldblatt's hypertension) has also been noted (Sikiric et al., 1993b). However, the mechanism remains elusive. A complex interaction with adrenergic and dopaminergic systems has been reported (Sikiric et al., 1997a). The release of this pentadecapeptide from somatosensory neurones is suggested, based on experiments with neurotoxin capsaicin, in a nociception assay (Sikiric et al., 1993a) and nasal (Kalogjera et al., 1997) and intestinal (Sikiric et al., 1996a) lesions studies. Since neuropeptides originating from the afferent sensory neurones in the vicinity of the microvessels are involved in the regulation of the release of the endothelium-derived mediators (Whittle et al., 1992), it was reasonable to investigate whether the effects of this novel stomach pentadecapeptide, BPC, could be a result of its interaction with nitric oxide (NO).

Extension of NO studies (usually limited to blunted generation of NO) and investigation of the influence of this novel gastric pentadecapeptide, under conditions of both a blunted generation of NO and NO substrate application, appear to be a rather logical approach in light of the widely suggested importance of NO as an essential signalling system in the gastrointestinal and cardiovascular systems (for review, see Moncada et al., 1991; Whittle et al., 1992). Bearing in mind the recognized dual role of NO, both an inhibition and an uncontrolled excess of NO would lead to significant damage (Lopez-Belmonte et al., 1993; Whittle et al., 1992). A beneficial effect was suggested for L-arginine, the precursor of NO (for review see Whittle et al., 1992), but agents which liberate NO are ulcerogenic when given in the higher doses (Lopez-Belmonte et al., 1993). L-arginine analogues and NO generation inhibitors are consistently claimed to be ulcerogenic (for review see Whittle et al., 1992) but bicarbonate secretion could be enhanced by these agents (Takeuchi et al., 1993; Takeuchi and Okabe, 1994), an effect also associated with an increase in systemic blood pressure (Takeuchi and Okabe, 1994). Finally, besides being essential for blood pressure maintenance, induction of NO synthase seems to be responsible for severe shock (Moncada et al., 1991). Thus, this investigation could help to further elucidate a variety of NO interactions.

Therefore, in addition to studying the interplay of NO/BPC 157 in the maintenance of stomach mucosal integrity, the effect of BPC 157 application on the blood pressure changes induced by a potent endothelial NO synthesis inhibitor N^G-nitro-L-arginine methylester (L-NAME) (Whittle et al., 1992) and a NO precursor, L-arginine (Moncada et al., 1991), are an additional focus in the present report. In addition, using the biochemical techniques described before by Whittle et al. (1992), the influence of this pentadecapeptide (along with L-arginine or L-NAME, given alone, or in combination) on the activity of NO synthase in homogenate supernatants of gastric mucosa from the rat stomach was investigated. To emphasize the experimental rationale for the present study, the NO system has to date not challenged by simultaneously testing the effect of an agent on the changes induced by L-arginine as well as L-NAME, given both separately and together, even though this dual activity of the NO system is widely recognized (for review, see Moncada et al., 1991; Whittle et al., 1992), at least from a theoretical point of view.

2. Materials and methods

2.1. Drugs

The pentadecapeptide BPC157 (GlyGluProProProGlyLysProAlaAspAspAlaGlyLeuVal, M.W. 1419), a partial sequence of human gastric juice peptide BPC (Sikiric et al., 1993a,b,c, 1994, 1996a,b), is freely soluble in water at pH 7.0 and in saline and was prepared as described before (Sikiric et al., 1993a,b,c, 1994, 1996a,b, 1997a,b,c; Seiwerth et al., 1997; Grabarevic et al., 1997). Peptide with

99% high-pressure liquid chromatography (HPLC) purity (1-des-Gly peptide as impurity), dissolved in saline, was used in all of the experiments (Sikiric et al., 1993a,b,c, 1994, 1996a,b, 1997a,b,c; Seiwerth et al., 1997; Grabarevic et al., 1997). L-NAME, L-arginine, D-arginine (Sigma, St. Louis, MO, USA) were dissolved in saline as described before (Takeuchi et al., 1993).

2.2. Animals

Male Wistar rats, 250–280 g body weight, were used for all of the experiments.

2.3. Ethanol model

Ethanol (96%) was given i.g. in a dose of 1.0 ml/rat as previously described (Sikiric et al., 1993b, 1994, 1996a) and the animals were euthanized 60 min after ethanol. Pentadecapeptide BPC 157 (10 μg/kg or 10 ng/kg i.p.) was given simultaneously with ethanol. L-NAME (5 mg/kg), L-arginine (200 mg/kg) and D-arginine (200 mg/kg) were given intravenously in the tail vein, alone or in combination, 5 min before ethanol or pentadecapeptide application. Controls received simultaneously the same volume of saline (5 ml/kg i.p. or 1 ml/kg i.v.). Immediately after the euthanasia, the stomach was removed and the lesions were assessed by naive observers as described before (i.e., photographed and lesion areas assessed morphometrically (using a computer program SFORM, VAMS, Zagreb, Croatia)) (Sikiric et al., 1993b, 1994, 1996a,b, 1997a,b). Representative sections of the stomach were processed for further histologic analysis as described before (Sikiric et al., 1993b, 1994, 1996a,b, 1997a,b,c).

2.4. Blood pressure assay

Mean systemic arterial blood pressure was assessed continuously for a 90 min period after drug medication by means of a cannula in the right carotid artery connected to a pressure transducer (Hellige, Germany) and a chart recorder. Rats were anaesthetized with urethane (0.6 g/kg i.p., 0.3 g/kg s.c.). 10 min after the start of surgery, BPC 157 (10 μg/kg or 10 ng/kg), L-NAME (5 mg/kg), L-arginine (200 mg/kg), and D-arginine (200 mg/kg) were given intravenously, alone or in combination, through a cannula inserted into the jugular vein. In pretreatment studies, based on our previous experiments, BPC 157 was applied 15 min before L-NAME or L-arginine application. In posttreatment studies this pentadecapeptide was given 10 min after L-NAME medication, corresponding to the time of the maximal L-NAME-induced blood pressure increase. L-arginine (or D-arginine) was given simultaneously with, or alternatively, 10 min after L-NAME administration. Controls received simultaneously an equivolume of saline (1 ml/kg i.v.). The blood pressure changes, which were originally estimated in mmHg, are expressed as the areas under the curve (AUC) (cm^2) (Campbell and Machin, 1995) (1 cm *y*-axis represents 10 mmHg, 1 cm *x*-axis represents 1 min) and they were assessed for each experimental group.

2.5. Tissue preparation, assay of NO and effect of BPC 157

Considering the gastric mucosa origin of the novel stomach pentadecapeptide BPC 157 (Sikiric et al., 1993a,b,c, 1994, 1996a,b, 1997a,b,c; Seiwerth et al., 1997; Grabarevic et al., 1997), its possible influence on the activity of NO synthase was investigated in homogenate supernatants of gastric mucosa from the rat stomach, using the same biochemical techniques described before by Whittle et al. (1992). Briefly, after overnight starvation and no water withdrawal, under urethane (0.6 g/kg i.p., 0.3 g/kg s.c.) anaesthesia, the abdominal aorta was cannulated and the stomach was washed free of blood with isotonic saline 15 ml/min for 5 min. After mucosal tissue was stripped away from underlying muscle on ice, homogenisation for 15 s on ice was carried out using a homogeniser (UVIS-1601, Shimitzu, Japan) in 3 volumes of a buffer containing HEPES (10 mM), sucrose (0.32 M), EDTA (0.1 mM), dithiothreitol (1 mM), soya bean trypsin inhibitor (10 μg/ml), leuptin (10 μg/ml) and aprotinin (2 μg/ml). Centrifugation of the homogenates was carried out at $10{,}000 \times g$ for 5 min (5°C) and the supernatant was stored on ice for up to 2 h.

For NO synthesis measurement, the oxidation of oxyhaemoglobin to methaemoglobin by NO was monitored spectrophotometrically. As in the procedure of Whittle et al. (1992), the difference between absorption at 401 and 421 nm was continuously monitored with a dual-wavelength spectrophotometer (Contron), using a band width of 5 nm, at 37°C. The incubate (500 μl) contained 1.6 μM oxyhaemoglobin, 1 mM $MgCl_2$, 40 mM potassium phosphate pH 7.2, 200 mM $CaCl_2$ and up to 20% (v/v) of the enzyme extract. For the calculation of the rate of NO formation, the absorption coefficient of methaemoglobin for the wavelength pair 401 minus 421 nm (77200 m^{-1} in the spectrophotometer) was used. For initiation of NO synthesis, besides NADPH (100 μM), which has been shown to be necessary (Whittle et al., 1992), L-arginine (100 μM) (D-arginine 100 μM was not effective) or pentadecapeptide BPC 157 (100 μM) addition was added. In other experiments, L-NAME (100, 500, 1000 μM) was given together with L-arginine or BPV 157. Alternatively, L-arginine (100 μM) and pentadecapeptide BPC 157 (100 μM) were given together.

2.6. Statistical analysis

Kolmogorov–Smirnov test was performed for estimation of the normality of the data distribution. Further statistical analyses were performed by means of analysis of

variance (ANOVA) and/or Kruskall–Wallis test, Student–Newman–Keuls, Dunn's and Dunnett's tests. Blood pressure changes were estimated using AUC-method (area under curve, cm^2) to avoid the type 1 error (false-positive differences, due to the large number of measurements). Differences of 0.05 or less were considered to be statistically significant.

3. Results

Methodologically, given the complex dual activity of NO (Moncada et al., 1991; Whittle et al., 1992), investigation of the various changes induced by BPC 157 added together with agents that affect the NO system in opposite ways, a simple way to assess the efficacy of the test drug. Such a protocol has not been previously applied in NO studies.

3.1. Ethanol lesions

Applied as before (Sikiric et al., 1993b, 1994, 1996a), this novel pentadecapeptide alone significantly prevented the otherwise severe gastric lesions screen in ethanol-treated control rats. In ethanol damaged rats pretreated with L-NAME, the same extent of lesions as in control was observed. But, if L-NAME was applied before BPC 157, the salutary effect of pentadecapeptide was attenuated (Fig. 1). L-arginine alone strongly reduced ethanol lesions. When it was given before the pentadecapeptide, the lesions remained the same as in the rats treated with pentadecapeptide alone (Fig. 1). The combined administration of L-NAME and L-arginine (D-arginine was not effective) led to lesions similar to those seen in the controls. When BPC 157 was applied after L-NAME + L-arginine combination, the salutary effect of the higher (μg) dose was markedly attenuated, whereas the effect of the lower (ng) dose was completely inhibited (Fig. 1). The microscopical observations were in line with macroscopically observed differences.

3.2. Blood pressure assay

The pentadecapeptide alone did not affect blood pressure. A prompt (maximum already after 10 min) and sustained elevation of blood pressure, in line with other studies, was produced by administration of L-NAME and the increase lasted for the entire 90-min-test period.

3.2.1. L-NAME / BPC157

When administered as pretreatment, a rather consistent beneficial activity was observed. After prophylactic administration of the novel pentadecapeptide before L-NAME the blood pressure increase was markedly reduced, particularly 10–65 min after L-NAME administration in the group treated with the higher dose of BPC 157 (means ± SD, mmHg, $140 \pm 16/119 \pm 26$ versus $181 \pm 21/150 \pm 2.2$ control) (Fig. 2).

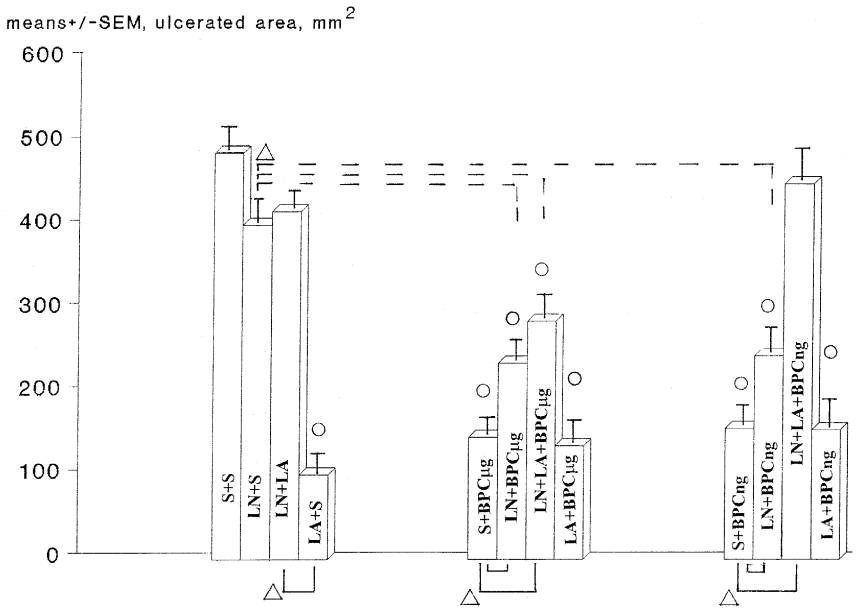

Fig. 1. Ethanol lesions (lesion areas, assessed morphometrically, means ± SEM, mm^2). 60 min after injury induction (96%, 1 ml i.g./rat). An equal volume of saline (S) was given simultaneously with the agents. L-NAME (LN) 5.0 mg/kg, L-arginine (LA) 200 mg/kg, given i.v. alone (LN + S, LA + S) or in combination (LN + LA), 5 min before ethanol. Pentadecapeptide BPC 157 10 μg or 10 ng/kg b.w. i.p. simultaneously with ethanol (i.e. 5 min after saline (S + BPC μg, S + BPC ng) or NO agent(s) (LN + BPC μg, LN + BPC ng, LA + BPC μg, LA + BPC ng, LN + LA + BPC μg, LN + LA + BPC ng) administration)). Controls received 5 ml/kg i.p. and 1 ml/kg i.v. of saline (S + S). $n = 8–12$ rats per each experimental group. (○) $P < 0.05$, at least versus control (S + S), (△) $P < 0.05$, at least versus corresponding groups.

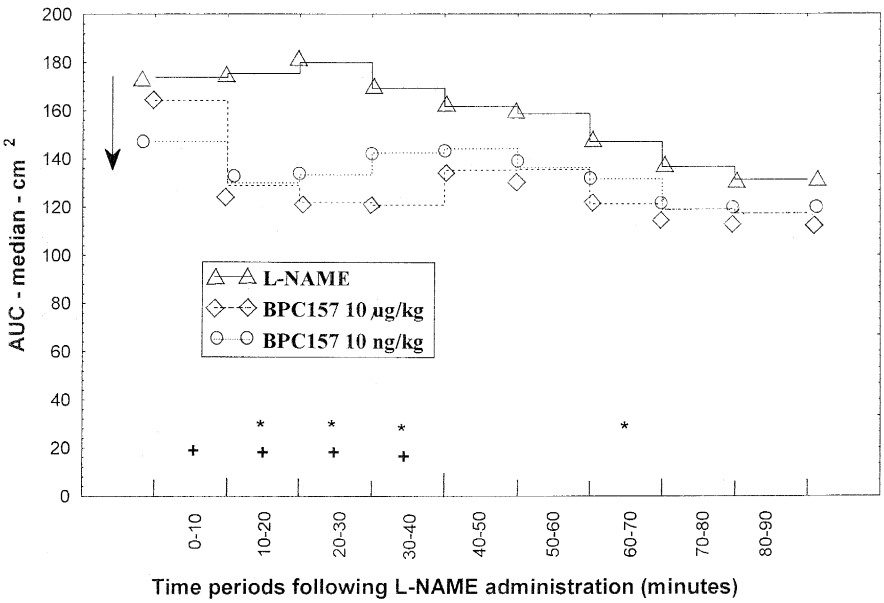

Fig. 2. Blood pressure. Prophylactic application (↓). Area under curve (median, cm^2). BPC 157 (10 μg or 10 ng/kg b.w. i.v.) or saline (control) (1 ml/kg i.v.) given 15 min before (↓) application of L-NAME (5.0 mg/kg b.w. i.v.) (time = 0). 90 min observation period. (★) $P < 0.05$, at least, BPC 157 μg + L-NAME versus L-NAME, (+) $P < 0.05$, at least, BPC 157 ng + L-NAME versus L-NAME. 8–12 rats per experimental group.

Concerning the salutary effect of this pentadecapeptide as a posttreatment, when it was applied at the time of the maximal increase in blood pressure, a significant decrease in blood pressure was clearly seen (10–45 min following the higher dose of BPC/134 ± 45/124 ± 33 mmHg versus control 181 ± 21/163 ± 6/mmHg, 5–20 min after the lower dose of BPC/143 ± 9/134 ± 25 mmHg versus control 176 ± 23/178 ± 25/mmHg) (Fig. 3).

3.2.2. L-arginine / L-NAME and L-arginine / BPC157

Applied 10 min after L-NAME, L-arginine (but not D-arginine) decreased blood pressure (Fig. 3). L-arginine

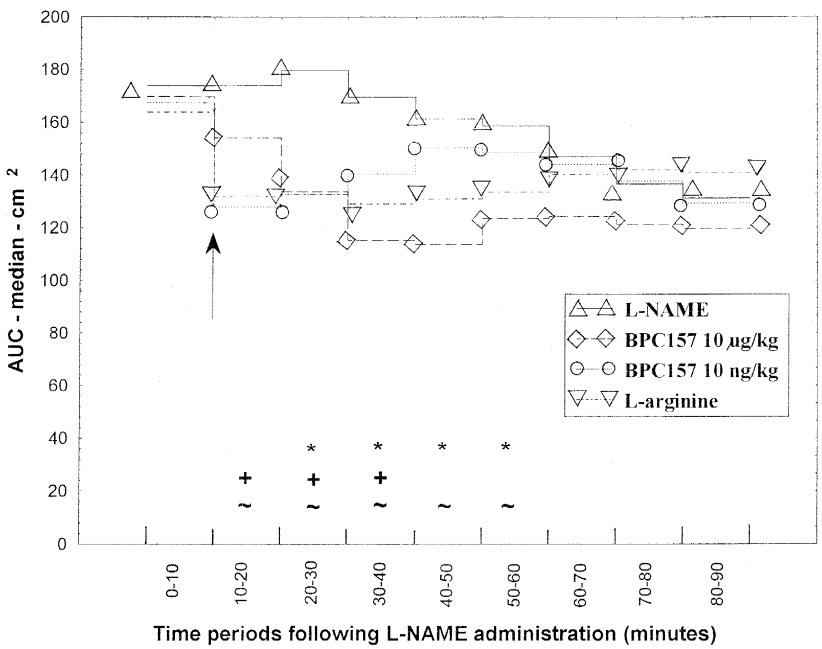

Fig. 3. Blood pressure. Therapeutic medication (↑). Area under curve (median, cm^2). BPC 157 (10 μg or 10 ng/kg b.w. i.v.) or L-arginine (200 mg/kg b.w. i.v.) were given (↑) 10 min after L-NAME 5.0 mg/kg b.w. i.v. (time = 0). Pentadecapeptide BPC 157 or L-arginine medication at the estimated maximal blood pressure. Controls received simultaneously an equal volume of saline (1 ml/kg i.v.). 90 min observation period. (★) $P < 0.05$, at least, L-NAME + BPC 157 μg versus L-NAME, (+) $P < 0.05$, at least, L-NAME + BPC 157 ng versus L-NAME, (~) $P < 0.05$, at least, L-NAME + L-arginine versus L-NAME. 8–12 rats per experimental group.

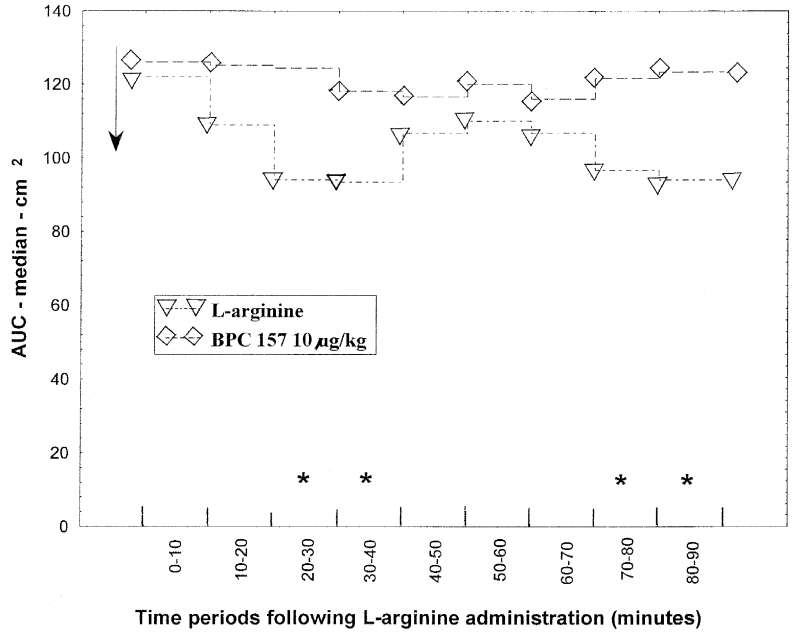

Fig. 4. Blood pressure. Prophylactic application (↓). Area under curve (median, cm^2). BPC 157 (10 μg/kg b.w. i.v.) or saline (control) (1 ml/kg i.v.) was given (↓) 15 minutes before administration of L-arginine (200 mg/kg b.w. i.v.) (time = 0). 90 min observation period. (★) $P < 0.05$, at least, BPC 157 + L-arginine versus L-arginine. 8 rats per experimental group.

alone caused a moderate decrease in blood pressure. This decrease was markedly prevented by prior BPC 157 application (Fig. 4).

3.2.3. L-NAME + L-arginine / BPC157

The combined administration of L-NAME and L-arginine (D-arginine was not effective) did not cause a blood pressure increase (Fig. 5). However, when BPC 157 was given after L-NAME + L-arginine-combination (i.e., at the same time as before in the case when L-NAME was given alone), its previously clear effect disappeared.

3.3. Assay of NO in gastric mucosa from stomach rat tissue homogenates and the influence of the pentadecapeptide BPC 157

L-arginine (but not D-arginine) induced the generation of NO by gastric mucosa from stomach rat tissue ho-

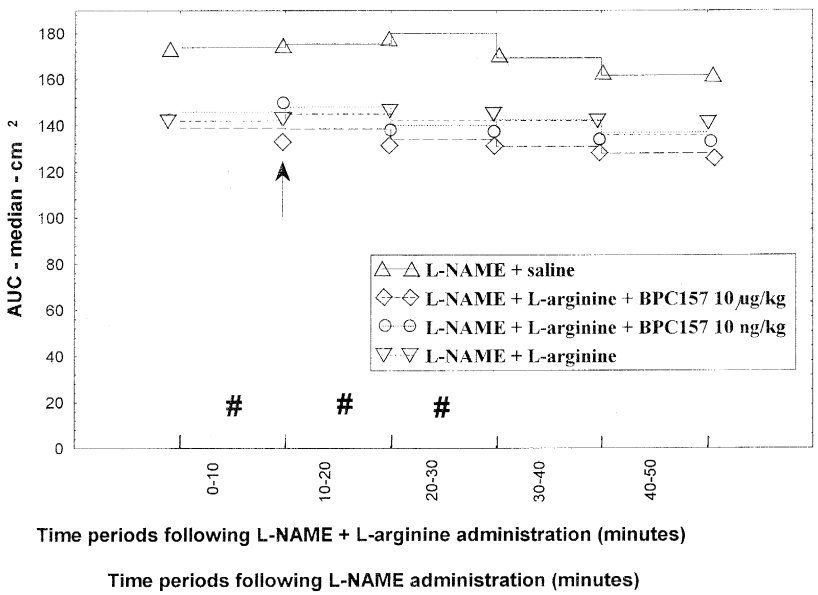

Fig. 5. Blood pressure. The effect of the pentadecapeptide BPC 157 after combined administration of L-NAME and L-arginine. Area under curve (median, cm^2). BPC 157 (10 μg or 10 ng/kg b.w. i.v.) or saline (control) (1 ml/kg i.v.) was given (↑) 10 min after administration of L-NAME 5.0 mg + L-arginine 200 mg/kg b.w. i.v. (time = 0). L-NAME 5 mg/kg b.w. i.v. application at the time 0. 90 min observation period. (#) $P < 0.05$, at least, L-NAME versus other groups. BPC157-groups versus L-NAME + L-arginine, $P > 0.05$. 8–12 rats per experimental group.

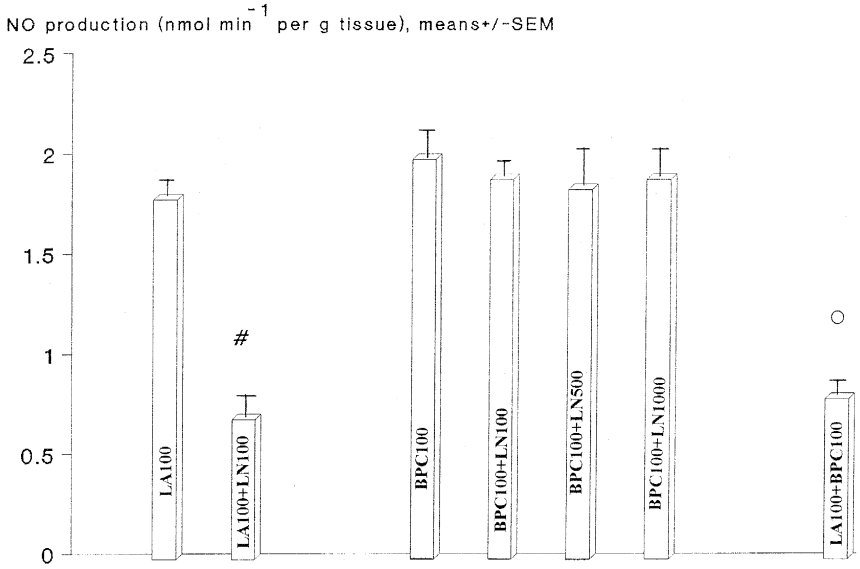

Fig. 6. Formation of nitric oxide (NO) following addition of L-arginine (100 μM) (LA100) and pentadecapeptide BPC 157 (100 μM) (BPC100) to homogenate supernatants of rat gastric mucosa. NO production (nmol min^{-1} per g tissue), was assessed spectrophotometrically over a 10 min period as the oxidation of oxyhaemoglobin to methaemoglobin. Inhibition of NO formation was attempted by incubation with L-NAME (100, 500, 1000 μM) (LA100 + LN100, BPC100 + LN100, BPC100 + LN500, BPC100 + LN1000). NO-synthesis was blunted when the pentadecapeptide BPC 157 (100 μM) and L-arginine (100 μM) (BPC100 + L-arginine 100) were combined. Means ± SEM of 8 experiments. (#) $P < 0.05$, at least, versus L-arginine, (○) $P < 0.05$, at least, versus BPC 157 or L-arginine.

mogenates (Fig. 6). This effect was clearly inhibited by the addition of L-NAME. Interestingly, pentadecapeptide BPC 157, given in the same dose as L-arginine, induced a comparable generation of NO. But, unlike L-arginine, its effect could not be inhibited by L-NAME, although even fivefold or tenfold higher doses were also used. NO synthesis was blunted when the pentadecapeptide BPC 157 and L-arginine were combined.

The values obtained for the NO-generating agents correlated fairly well with previously reported values (Whittle et al., 1992).

4. Discussion

Theoretically, the use of agents that positively or negatively affect the NO system, given separately or in combination, would be advantageous for a through determination of the possible relation of NO with other agents and systems. Although to date this approach has not been used extensively (for review, see, e.g., Moncada et al., 1991; Whittle et al., 1992), it seemed to us that this approach could be particularly helpful against the background of the suggested dual role of NO.

The known positive effects of pentadecapeptide BPC 157 on ethanol-induced gastric lesions and blood pressure maintenance (Sikiric et al., 1993b, 1994, 1996a) were challenged with a combination of L-NAME, a competitive inhibitor of endothelium NO generation and the NO precursor, L-arginine (for review, see, e.g., Moncada et al., 1991; Whittle et al., 1992) given separately and/or together. As these agents affect NO synthase in both ways (positively or negatively), a possible role of this pentadecapeptide could be either excluded or, at least partly, proven. It was shown that pentadecapeptide could reverse the changes induced by either L-NAME or L-arginine, given separately, toward the control basal values. After L-NAME and L-arginine, when applied together, its effect was blunted. In homogenate supernatants of gastric mucosa from the rat stomach, like L-arginine (Whittle et al., 1992), this pentadecapeptide induce NO formation, but its effect was not blunted by L-NAME addition. NO synthesis was blunted when the pentadecapeptide BPC 157 and L-arginine were combined.

In general similar findings were obtained in these two distinctive in vivo models, when the same doses of the tested agents, pentadecapeptide BPC 157 and NO agents, were used. Consequently, it seems likely that the effects elicited by the combination of pentadecapeptide BPC 157 and agents negatively (L-NAME) or positively (L-arginine) affect NO synthesis are not random, indicating a specificity of action, particularly in the light of the generation of NO in stomach mucosa induced by pentadecapeptide BPC 157 in rat tissue homogenates.

As an intriguing point, pentadecapeptide's original beneficial effect could not be restored after L-NAME + L-arginine combination. Moreover, after addition of L-arginine to L-NAME, its effect was further attenuated (gastric lesions) and almost completely disappeared (blood pressure). Thus, concerning the NO system, it seems that combining a stimulatory (L-arginine) and an inhibitory (L-NAME) agent can blunt the activity of this pentade-

capeptide. But, more attention should be probably focused to the L-arginine effect, an aspect which to date seems to be particularly neglected in investigations of NO-related drugs effects (for review, see Moncada et al., 1991; Whittle et al., 1992).

Since it has been shown, in both in vivo and in vitro experiments, that the pentadecapeptide BPC 157 can override the effect of L-NAME (an essential point indicated for L-arginine (for review, see Moncada et al., 1991; Whittle et al., 1992)), a particular interaction with the NO system seems to be possible. Assuming that drugs such as L-NAME inhibit NO synthase by competition with L-arginine (for review, see Moncada et al., 1991; Whittle et al., 1992), a similar competition with the pentadecapeptide could be speculated. The findings of our in vitro experiments seem to be consistent with this notion. NO generation was attenuated when L-arginine and pentadecapeptide BPC 157 were combined, although when given separately, both of them initiated NO-synthesis. Therefore, this competition between pentadecapeptide L-arginine appears to be possible in vivo as well, particularly if NO synthase availability is limited for example, when it has already been inhibited markedly by L-NAME. This could be explained by the additional inhibition of the activity of the pentadecapeptide when L-arginine was added to L-NAME, as consistently seen in both gastric lesions and blood pressure assays.

Seen from the viewpoint of the pentadecapeptide BPC157/L-arginine/NO system, the finding of not more than a limited competition, especially with L-arginine-analogues (such as L-NAME), could be only indicated in the opposite case (a situation, mostly noted to date in NO studies and regularly interpreted as a close relationship with NO system (for review, see Moncada et al., 1991; Whittle et al., 1992)). In this, contrary to common understanding, the observation that the effect of agent(s) can be blunted by L-arginine analogues and restored by the addition of L-arginine to L-arginine analogues (such as L-NAME) (for review, see Moncada et al., 1991; Whittle et al., 1992) should not necessarily be taken in favour of an essential competition with L-arginine itself. Namely, for there to be competition between the reference agents and the L-arginine analogue (i.e., agent + L-arginine analogue) it is essential to see an inhibition of these agents' effect (for review, see Moncada et al., 1991; Whittle et al., 1992). In this combination (i.e., standard agent + L-arginine + L-arginine analogue), L-arginine evidently could easily operate (for review, see Moncada et al., 1991; Whittle et al., 1992). Obviously, a rather unopposed action of L-arginine itself, along with a lack of direct studies (e.g., agent(s) + L-arginine), could be hardly evidence for a close interaction between the agents studied to date and L-arginine, a NO precursor. Thus, this discrepancy could suggest that a possible interaction with the NO system, as has been pointed out, is apparently limited. In contrast and in line with the suggestion about the likely competition between this pentadecapeptide and L-arginine, the evidence for a BPC157/L-arginine/NO system interaction appears to be more complete, obtained in both in vitro and in vivo studies. In our in vitro studies, NO generation was attenuated when these agents were combined and excess of NO generation prevented. Interestingly, in the blood pressure assay, BPC 157 application markedly prevented the L-arginine-induced blood pressure decrease. Since in vitro experiments, BPC 157-induced NO formation was not inhibited by the addition of L-NAME, although L-NAME was given also in a dose which was fivefold or tenfold higher than that needed for inhibition of the effect of L-arginine, it is likely that BPC 157 has a more marked and/or different effect on NO than L-arginine. This supposition seems to be supported by the comparable anitulcerogenic effect of these agents, L-arginine and BPC 157. But, when challenged with the same dose of L-NAME, the L-arginine effect was completely abolished in ethanol-injured rats, whereas the BPC 157 effect was only attenuated.

Theoretically, the possibility that BPC, which is formed constitutively in the gastric mucosa (body) and which is present in gastric juice (Sikiric et al., 1993a,b,c, 1994, 1996a,b, 1997a,b; Seiwerth et al., 1997; Grabarevic et al., 1997; Veljaca et al., 1995a), competes effectively with both L-arginine analogues (such as L-NAME) and L-arginine could have some physiologic importance. Given the suggested significance of NO synthase and the basal formation of NO in stomach mucosa, which is greater than that seen in other tissues (i.e., lung, liver) (Whittle et al., 1992), it is not entirely unexpected that a novel stomach pentadecapeptide with marked stability in gastric juice (Sikiric et al., 1993b; Veljaca et al., 1995a,b) and no similarity with known peptides (Sikiric et al., 1993b) could exert such effects. Because calcium-and NADPH-dependent NO synthase is present in gastric tissue in the microvascular endothelium and probably in other gastric mucosal cells (Whittle et al., 1992), the precise site of pentadecapeptide interaction remains to be further determined. Likewise, based on the close similarity of the determination of NO synthase in gastric and other tissues (i.e., vascular, lung and liver tissue) (Whittle et al., 1992), this pentadecapeptide would probably have a similar activity in other organs as well. This could provide a basis for multiple effects (Sikiric et al., 1993a,b,c, 1994, 1996a,b, 1997a,b; Seiwerth et al., 1997; Grabarevic et al., 1997) in addition to the suggested protective role of this pentadecapeptide in the stomach and other organs. In view of the dual effects of the drugs affecting NO synthase (Lopez-Belmonte et al., 1993) and the essential signalling role of NO in gastric mucosal integrity and blood pressure maintenance (for review see Moncada et al., 1991; Whittle et al., 1992), a possible modulatory role of this pentadecapeptide could be advantageous.

Whether this novel pentadecapeptide has such a modulatory role and/or whether it interacts with the NO system at all, remains to be seen. The effect of L-NAME appears

to be mostly limited to the endothelium (i.e., Moncada et al., 1991). The amount of NO precursors L-arginine in the endothelium could be limited in some pathologic circumstances (Moncada et al., 1991) and L-arginine has no direct vasodilator activity (Rees et al., 1990). In gastric ethanol injury, the endothelium is the first preferential target for ethanol damage (Szabo et al., 1985). Along with an increase in systemic blood pressure, vasoconstriction of several vascular beds, i.e., mesenteric and renal beds, produced by systemic administration of L-arginine analogues, given to inhibit generation of NO (Moncada et al., 1991), would be certainly harmful, specially for the gastric mucosa and its endothelium. These tissues need a permanent oxygen and blood supply (Menguy et al., 1974; Szabo et al., 1985; Holzer, 1992). Thus, when there is blunted NO release, together with an increase in blood pressure due to systemic vasoconstriction, a strong systemic salutary action is obviously needed to prevent the local consequences of intragastrical application of ethanol. Endothelium protection, which is recognized as being essential to prevent in careful studies of ethanol-induced gastric damage, has been implicated also as an essential factor in wider organoprotection activity (Szabo et al., 1985). As mentioned, the vascular endothelium is capable of modulating vascular tone, in part by the generation of a substance such as the endothelium-derived relaxing factor (EDRF), which has been identified as NO (Moncada et al., 1991). The pentadecapeptide BPC 157, has been shown to protect the endothelium, using the well-known Szabo's protocol of ethanol-induced gastric damage assessed with Monastral blue (Szabo et al., 1985; Sikiric et al., 1993b, 1994), and very recently its special cytoprotective role was further accentuated (Sikiric et al., 1997b). Likewise, BPC 157 is claimed to have beneficial effects even outside the gastrointestinal tract (Sikiric et al., 1993a,b,c, 1996a,b; Seiwerth et al., 1997; Grabarevic et al., 1997; Paré and Klucyznski, 1994; Veljaca et al., 1994a,b, 1995a,b; Bosnjak et al., 1994), including heart protection, following hypoxic and reoxygenation injury in the isolated guinea pig heart (Sikiric et al., 1993b; Bosnjak et al., 1994). Like many other gut peptides, BPC 157 is present in different organs (Sikiric et al., 1993b) and its possible special role in the interaction with the endothelium derived NO system should be further investigated.

Intriguingly, unlike L-arginine and its analogues (Moncada et al., 1991) BPC 157 alone, applied even in the higher dosages (i.e., mg/kg) could not influence basal blood pressure values (Sikiric et al., 1993b). However, it was shown that the following application of this pentadecapeptide, an otherwise fatal fall in blood pressure in rats with severe hemorrhagic shock, could be reversed toward normal values and survival markedly increased (Sikiric et al., 1993b). It is also likely that BPC 157 can inhibit the effect of a calcium ionophore A23187, which is known to stimulate NO release (Moncada et al., 1991). For instance, the pentadecapeptide could dose-dependently reduce the release of leukotriene B_4 in human blood stimulated by A23187 (Veljaca et al., 1994a,b, 1995b). Besides this, BPC 157 could also inhibit other inflammatory mediators, i.e., myeloperoxidase, tromboxane B_2 (Veljaca et al., 1994a,b, 1995b). This action should probably also be considered because NO can also be produced by the inducible NO synthase of activated macrophages and neutrophils (Moncada et al., 1991). NO is destroyed by superoxide anions and inactivation of superoxide anions protects and prolongs the action of NO (e.g., Whittle et al., 1992). The pentadecapeptide BPC 157 has been shown to have scavenging properties and to provide protection against free radical-induced lesions (Sikiric et al., 1993b, 1994). Finally, NO synthase activity appears to be partially involved in the gastric cytoprotection (Ko and Cho, 1994) and blockade of both capsaicin-sensitive afferent neurones and NO synthesis is needed to produce gastrointestinal damage (Holzer, 1992). Besides this, capsaicin-sensitive afferent neurons control the activity of sympathetic vasoconstrictor nerves (Holzer, 1992). As mentioned, compelling evidence was recently provided for a complex synergistic interaction between the beneficial effects of BPC 157 and peptidergic sensory afferent neurone activity in various experimental paradigms (e.g., nociception, gastrointestinal lesions, damaged nasal mucosa) (Sikiric et al., 1993a,b, 1996a; Kalogjera et al., 1997). The regulation of the release of the endothelium-derived mediators by neuropeptides originating from afferent sensory neurones in the vicinity of the microvessels is also suggested (Whittle et al., 1992).

In summary, based on the generally accepted importance of NO as an essential signalling agent in the gastrointestinal and cardiovascular systems (for review, see Moncada et al., 1991; Whittle et al., 1992) and its possible dual role, we studied the influence of a novel pentadecapeptide, BPC 157, under conditions of a blunted generation of NO and NO substrate application. It was clearly shown that BPC 157 could interfere with the effect of NO agents in both gastric mucosal integrity and blood pressure maintenance in a specific way. In studies using homogenate supernatants of rat gastric mucosa, the pentadecapeptide BPC 157 had an effect on NO generation that was more pronounced and/or different from the effect of L-arginine. Very recently, similar findings have been reported for chickens (Grabarevic et al., 1997). Pulmonary hypertension syndrome or ascites in broilers (described as an accumulation of the oedematous fluid within the abdominal cavity as a consequence of heart failure syndrome (Scheele et al., 1991)) was induced by chronic application of L-NAME, whereas L-arginine had a detrimental effect specially on the myocardium. Interestingly, the pentadecapeptide BPC 157 could markedly prevent the appearance of lesions in various organs (Grabarevic et al., 1997). Together, considering that the birds, unlike mammals, cannot de novo synthesise L-arginine and their plasma concentration of this amino acid is directly correlated with

its dietary intake (Tamil and Ratner, 1963), it seems that these beneficial effects of BPC 157 are a common phenomenon. It remains, however, to fully established whether this interference is due to a parallel and/or interconnected action. Similarly, whether this pentadecapeptide can interfere in the same way with the effects of a NO donor would be an interesting topic for further studies. Besides, both the protective effect (low dose) and ulcerogenic effect (high dose) of the agents which liberate NO are suggested to be specifically NO-mediated, although attempts to prove otherwise have not been made (Lopez-Belmonte et al., 1993). Likewise, the extension of this study to the effect of other agents in response to challenge with L-arginine and L-arginine analogues appears an additional interesting goal.

Acknowledgements

This study was subsidized by grants from the Ministry of Science of Republic of Croatia and Pliva, Zagreb, Croatia.

References

Bosnjak, Z.J., Graf, B.M., Sikiric, P., 1994. Protective effects of newly isolated gastric peptide following hypoxic and reoxygenation injury in the isolated guinea pig heart. FASEB J. 8, A129.

Campbell, M.J., Machin, D. (Eds.), 1995. Medical Statistics. A Commonsense Approach. John Wiley&Sons, Chichester.

Grabarevic, Z., Tisljar, M., Artukovic, B., Bratulic, M., Dzaja, P., Seiwerth, S., Sikiric, P., Peric, J., Geres, D., Kos, J., 1997. The influence of BPC 157 on nitric-oxide agonists and antagonist induced lesions in broiler chicks. J. Physiol., in press.

Holzer, P., 1992. Peptidergic sensory neurons in the control of vascular functions: Mechanisms and significance in the cutaneous and splanchnic vascular beds. Rev. Physiol. Biochem. Pharmacol. 121, 50.

Kalogjera, L., Ries, N., Baudoin, T., Ferencic, Z., Protic, R., Pegan, B., 1997. Dose dependent protective effect of BPC 157 in capasaicin induced rhinitis in rats. Eur. Arch. Otorhynolaringol. 254, S9.

Ko, J.K.S., Cho, C.H., 1994. Involvement of nitric oxide in the adaptive cytoprotection of mild irritants against injury caused by ethanol. Digestion 55 (S2), 20.

Lopez-Belmonte, J., Whittle, B.J.R., Moncada, S., 1993. The actions of nitric oxide donors in the prevention or induction of injury to the rat gastric mucosa. Br. J. Pharmacol. 108, 73.

Menguy, R., Desbaillets, L., Masters, Y.F., 1974. Mechanism of stress ulcer: Influence of hypovolemic shock on energy metabolism in the gastric mucosa. Gastroenterology 66, 46.

Moncada, S., Palmer, R.M.J., Higgs, E.A., 1991. Nitric oxide: Physiology, pathophysiology and pharmacology. Pharmacol. Rev. 43, 109.

Paré, W., Klucyznski, J.M., 1994. The effect of new gastric juice peptide BPC on classic stress triad in stress procedure. Exp. Clin. Gastroenterol. 2, 234.

Rees, D.D., Palmer, R.M.J., Schulz, R., Hudson, H.F., Moncada, S., 1990. Characterization of three inhibitors of endothelial nitric oxide synthetase in vitro and in vivo. Br. J. Pharmacol. 101, 746.

Sandor, Z., Vincze, A., Szabo, S., 1996. The protective effect of a recently isolated gastric peptide in acute and chronic gastroduodenal injury. FASEB J. 10, A171.

Scheele, C.W., de Wit, W., Frankenhuis, M.T., Vereijken, P.F.G., 1991. Ascites in broilers. 1. Experimental factors evoking symptoms related to ascites. Poultry Sci. 70, 1069.

Seiwerth, S., Sikiric, P., Grabarevic, Z., Zoricic, I., Hanzevacki, M., Ljbanovic, D., Coric, V., Konjevoda, P., Petek, M., Rucman, R., Turkovic, B., Perovic, D., Mikus, D., Jadrijevic, S., Medvidovic, M., Tadic, T., Romac, B., Kos, J., Peric, J. and Kolega, Z., 1997. BPC 157 effect on healing. J. Physiol. (Paris), in press.

Sikiric, P., Gyires, K., Seiwerth, S., Grabarevic, Z., Rucman, R., Petek, M., Rotkvic, I., Turkovic, B., Udovicic, I., Jagic, V., Mildner, B., Duvnjak, M., Danilovic, Z., 1993a. The effect of pentadecapeptide BPC 157 on inflammatory, non-inflammatory, direct and indirect pain and capsaicin neurotoxicity. Inflammopharmacology 2, 121.

Sikiric, P., Petek, M., Rucman, R., Seiwerth, S., Grabarevic, Z., Rotkvic, I., Jagic, V., Mildner, B., Duvnjak, M., Lang, N., 1993b. A new gastric juice peptide, BPC-an overview of stomach/stress/organoprotection hypothesis and BPC beneficial effects. J. Physiol. (Paris) 87, 313.

Sikiric, P., Seiwerth, S., Grabarevic, Z., Rucman, R., Petek, M., Rotkvic, I., Turkovic, B., Jagic, V., Mildner, B., Duvnjak, M., Danilovic, Z., 1993c. Hepatoprotective effect of BPC 157, a 15-aminoacid peptide, on liver lesions induced by either restraint stress or bile duct and hepatic artery ligation or CCl_4 administration. A comparative study with dopamine agonists and somatostatin. Life Sci. 53, 291.

Sikiric, P., Seiwerth, S., Grabarevic, Z., Petek, M., Rucman, R., Turkovic, B., Rotkvic, I., Jagic, V., Duvnjak, M., Mise, M., Djacic, S., Separovic, J., Veljaca, M., Sallmani, A., Banic, M., Brkic, T., 1994. The beneficial effect of BPC 157, a 15 amino acid peptide BPC fragment, on gastric and duodenal lesions induced by restraint stress, cysteamine and 96% ethanol in rats. A comparative study with H2 receptor antagonists, dopamine promotors and gut peptides. Life Sci. 54, 63.

Sikiric, P., Seiwerth, S., Grabarevic, Z., Rucman, R., Petek, M., Jagic, V., Turkovic, B., Rotkvic, I., Mise, S., Zoricic, I., Gjurasin, M., Konjevoda, P., Separovic, J., Ljubanovic, D., Artukovic, B., Bratulic, M., Tisljar, M., Jurina, Lj., Buljat, G., Miklic, P., Marovic, A., 1996a. The beneficial effect of a novel pentadecapeptide BPC 157 on gastric lesions induced by restraint stress, ethanol, indomethacin and capsaicin neurotoxicity. Dig. Dis. Sci. 41, 1604.

Sikiric, P., Seiwerth, S., Grabarevic, Z., Rucman, R., Petek, M., Jagic, V., Turkovic, B., Rotkvic, I., Mise, S., Zoricic, I., Jurina, Lj., Konjevoda, P., Hanzevacki, M., Ljubanovic, D., Separovic, J., Gjurasin, M., Bratulic, M., Artukovic, B., Jelovac, N., Buljat, G., 1996b. Salutary and prophylactic effect of pentadecapeptide BPC 157 on acute pancreatitis and concomitant gastroduodenal lesions in rats. Dig. Dis. Sci. 41, 1518.

Sikiric, P., Mazul, B., Seiwerth, S., Grabarevic, Z., Rucman, R., Petek, M., Jagic, V., Turkovic, B., Rotkvic, I., Mise, S., Zoricic, I., Jurina, L.J., Konjevoda, P., Hanzevacki, M., Gjurasin, M., Separovic, J., Ljubanovic, D., Artukovic, B., Bratulic, M., Tisljar, M., Miklic, P., Sumajstorcic, J., 1997a. Pentadecapeptide BPC 157 interactions with adrenergic and dopaminergic systems in mucosal protection in stress. Dig. Dis. Sci. 42, 661.

Sikiric, P., Mikus, D., Seiwerth, S., Grabarevic, Z., Rucman, R., Petek, M., Jagic, V., Turkovic, B., Rotkvic, I., Mise, S., Zoricic, I., Peric, J., Konjevoda, P., Perovic, D., Jurina, L.J., Hanzevacki, M., Separovic, J., Gjurasin, M., Jadrijevic, S., Jelovac, N., Miklic, P., Buljat, G., Marovic, A., 1997b. Pentadecapeptide BPC 157, cimetidine, ranitidine, bromocriptine and atropine effect in cysteamine lesions in totally gastrectomized rats. A model study for cytoprotective studies. Dig. Dis. Sci. 42, 1029.

Sikiric, P., Seiwerth, S., Grabarevic, Z., Rucman, R., Petek, M., Jagic, V., Turkovic, B., Rotkvic, I., Mise, S., Zoricic, I., Perovic, D., Simicevic, V., Hanzevacki, J., Ljubanovic, D., Artukovic, B., Bratulic, M., Tisljar, M., Rekic, B., Gjurasin, M., Miklic, P., Buljat, G., 1997c. Pentadecapeptide BPC 157 positively affects both non-steroidal anti-inflammatory agents-induced gastrointestinal lesions and adjuvant arthritis in rats. J. Physiol. (Paris), in press.

Szabo, S., Trier, J.S., Brown, A., Schnoor, J., 1985. Early vascular injury

and increased vascular permeability in gastric mucosal injury caused by ethanol in the rat. Gastroenterology 88, 228.

Takeuchi, K., Okabe, S., 1994. Mechanisms underlying stimulation of gastric bicarbonate secretion by the NO synthase inhibitor L-NAME in rats. Digestion 55 (S2), 44.

Takeuchi, K., Ohuchi, T., Miyake, H., Niki, S., Okabe, S., 1993. Effect of nitric oxide synthase inhibitors on duodenal alkaline secretion in anaesthetized rats. Eur. J. Pharmacol. 231, 135.

Tamil, H., Ratner, S., 1963. A study of ornithine, citrulline and arginine synthesis in growing chick. Arch. Biochem. Biophys. 102, 259.

Veljaca, M., Lech, C.A., Pllana, R., Sanchez, B., Chan, K., Guglietta, A., 1994a. BPC-15 reduces trinitrobenzene sulfonic acid-induced colonic damage in rats. J. Pharmacol. Exp. Ther. 272, 417.

Veljaca, M., Pllana, R., Lesch, C.A., Sanchez, B., Chan, K., Guglietta, A., 1994b. Protective effect of BPC 157 on a rat model of colitis. Gastroenterology 106, 789.

Veljaca, M., Chan, K., Guglietta, A., 1995a. Digestion of h-EGF, h-TGF alpha and BPC-15 in human gastric juice. Gastroenterology 108, 761.

Veljaca, M., Lesch, C.A., Sanchez, B., Low, J., Guglietta, A., 1995b. Protection of BPC-15 on TNBS-induced colitis in rats: Possible mechanisms of action. Gastroenterology 108, 936.

Whittle, B.J.R., Boughton-Smith, N.K., Moncada, S., 1992. Biosynthesis and role of the endothelium-derived vasodilator, nitric oxide in gastric mucosa. Ann. N.Y. Acad. Sci. 664, 126.