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THE EFFECT OF PENTADECAPEPTIDE BPC 157 ON INFLAMMATORY, NON-INFLAMMATORY, DIRECT AND INDIRECT PAIN AND CAPSAICIN NEUROTOXICITY

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ABSTRACT

Sikirič P, Gyires K, Seiwerth S et al. The effect of pentadecapeptide BPC 157 on inflammatory, non-inflammatory, direct and indirect pain and capsaicin neurotoxicity. Inflammopharmacology. 1993;2; 121–127.

The anti-nociceptive effects of a newly synthesized pentadecapeptide coded BPC 157 (an essential fragment of new organoprotective gastric juice peptide BPC) was evaluated in comparison with aspirin and morphine reference standards, in various experimental models of indirect/direct nociception and neurotoxicity: writhing (acetic acid/magnesium sulphate), tail pinching, hot-plate, and capsaicin application. BPC 157 administered either in the ng or μg per kg range, intraperitoneally, significantly reduced the reactions in the writhing (inflammatory and non-inflammatory, prostaglandin-dependent and independent) and tail pinching tests. In the hot-plate test, unlike morphine, BPC 157 had no effect on normal animals. However, when given to capsaicin treated rats, BPC 157 strongly reduced capsaicin-allodynia, either given as pretreatment or once daily for 14 days after the capsaicin injection. This reduction in capsaicin's effect could not be obtained when BPC 157 was applied in the presence of established capsaicin-somatosensory neuron degeneration (application only on the 14th day after capsaicin), so it is possible that the effects of BPC 157 could be related specifically to the integrity of capsaicin-sensitive somatosensory neurons and their protection (e.g. primary afferent neurons having small-diameter somata and unmyelinated (C-) or thinly myelinated (Aδ-) fibres).

Keywords: pentadecapeptide BPC 157, essential fragment of organoprotective gastric juice peptide BPC, writhing (acetic acid/magnesium sulphate), tail pinching test, hot-plate test, inflammatory/non-inflammatory, indirect/direct nociception, capsaicin, allodynia, capsaicin-sensitive somatosensory neurons integrity/protection

INTRODUCTION

A profound anti-inflammatory, anti-pyretic and analgesic effect was observed for a new gastric peptide, code-named BPC, with marked organoprotective effects, recently isolated and characterized by our group [1–4], and a 15-amino acid fragment of this peptide (BPC 157), thought to be essential for the entire BPC activity, has been characterized [1–4]. The observed protective effects of the gastric peptide involve the hormones of adrenal, parathyroid, thyroid and ovarian glands [1–4]. Such protective actions have also been investigated independently and confirmed by others [5,

Durakovic, Paré, Gyires, unpublished symposium communication, Zagreb, 1992; unpublished communication, Milwaukee, 1993]. The present report investigates the influence of BPC 157 on different experimental models of nociception and neurotoxicity [6–17].

MATERIALS AND METHODS

BPC 157 preparation

BPC 157, a partial sequence of the natural peptide BPC (isolated from human gastric fluid), consists of 15 amino acids: Gly-Glu-en-Pro-Pro-Pro-Gly-Lys-Pro-Ala-Asp-Asp-Ala-Gly-Leu-Val. This pentadecapeptide was obtained [4] by stepwise condensation of fluorenylmethylchloroformate (Fmoc) protected amino acids, beginning first with valine, which was bonded onto a polymeric carrier (benzhydrilamino resin), using diisopropylcarbodiimide as a coupling reagent. In each step the Fmoc protective group was removed with piperidine. Thereafter the sequence of amino acids was built up until the synthesis was complete. Cleavage was effected by a mixture of trifluoroacetic acid/trifluoromethanesulphonic acid/anisole (2:17:52). The raw peptide mixture was purified on a high pressure liquid chromatography (HPLC) column 5 mm i.d., 150 mm length, using silica RP-18, gradient elution and a solvent system consisting of: 0.1% trifluoroacetic acid in water/acetonitrile (50/50 v/v). The resulting pentadecapeptide had minimum 95% purity and was used for the experiments.

Experimental procedures

NMRI Hannover mice (20 g b.w.) and albino Wistar rats (150 g b.w.), of either sex, were used.

Writhing

As a challenge, 0.6% acetic acid or 2% magnesium sulphate (both given as 0.2 ml/mouse i.p.) were used. BPC 157 (10 μg or 10 ng/kg, b.w. i.p.) was given either at the same time or 30 min before the challenge. Control mice received simultaneously 0.9% NaCl (5.0 ml/kg, b.w. i.p.). The number of wriths was continuously monitored (animals were in separate cages) for 5 min after the first movement.

Tail pinching test

A tail pinching test (pressure applied constantly on the proximal tail) was used on mice at 0, 5, 10, 15, 30, 45, 75 and 90 min, as well as 24 h after drug treatment, and the time for their reactions was calculated. The mice not reacting within 10 s were considered to be inactive. BPC 157 (10 μg or 10 ng/kg, b.w. i.p.), aspirin (100 mg/kg, i.p.), or 0.9% NaCl (5.0 ml/kg, b.w. i.p.) were given.

Hot-plate test

The usual hot plate temperatures (54.6°C mice, 55°C rats) were used. The animals were conditioned before the experimental procedure was begun, and only those reacting (jumping, licking the hind paws) within 13 s (mice) or 45 s (rats) were used. No time longer than 40 s (mice) or 120 s (rats) was used. BPC 157 (10 μg or 10 ng/kg, b.w. i.p.), morphine (2.5 mg/kg, b.w. s.c.) or 0.9% NaCl (5.0 ml/kg, b.w. i.p.) were given to mice 1 h before the test. Capsaicin, in a single dose of 125 mg/kg b.w., s.c. was given to rats which were tested 10, 11, 12, 13 and 14 days thereafter. BPC 157 (10 μg/kg, b.w., i.p.) was given either as pretreatment (1 h before the capsaicin), or simultaneously with capsaicin, and thereafter once daily, for 14 days, or once after the 14th day, 1 h before hot-plate test assessment.

In general, observers were unaware of the treatment given.

Statistical analysis

An analysis of variance (ANOVA), followed by Dunnett's test, Fisher's exact probability test, and Kruskall–Wallis test were used for analysis. A p value of 0.05 or less were considered to be statistically significant.

RESULTS

Writhing

In the acetic acid assay, a strong, dose-dependent reduction of movements was seen in BPC 157-treated animals (Figure 1). BPC 157 also seemed to be effective in reducing magnesium sulphate-induced movements, particularly when given with the challenge. The pentadecapeptide itself does not induce any writhing.

Tail pinching test

In the 90 minute study, both aspirin and BPC 157 increased the number of non-responding mice, and prolonged the time taken before a response was made. Moreover, compared with aspirin, the BPC 157 effect was more pronounced (Figure 2). This effect was not present 24 h thereafter (data not shown).

Hot-plate test

Unlike morphine, no effect was noted for BPC 157 in normal mice. However, when given to capsaicin-treated rats, BPC 157 given either as pretreatment or once daily for 14 days after capsaicin reduced the capsaicin-allodynia (Figure 3). This effect could not be obtained when BPC 157 was given only on the 14th day after capsaicin treatment.

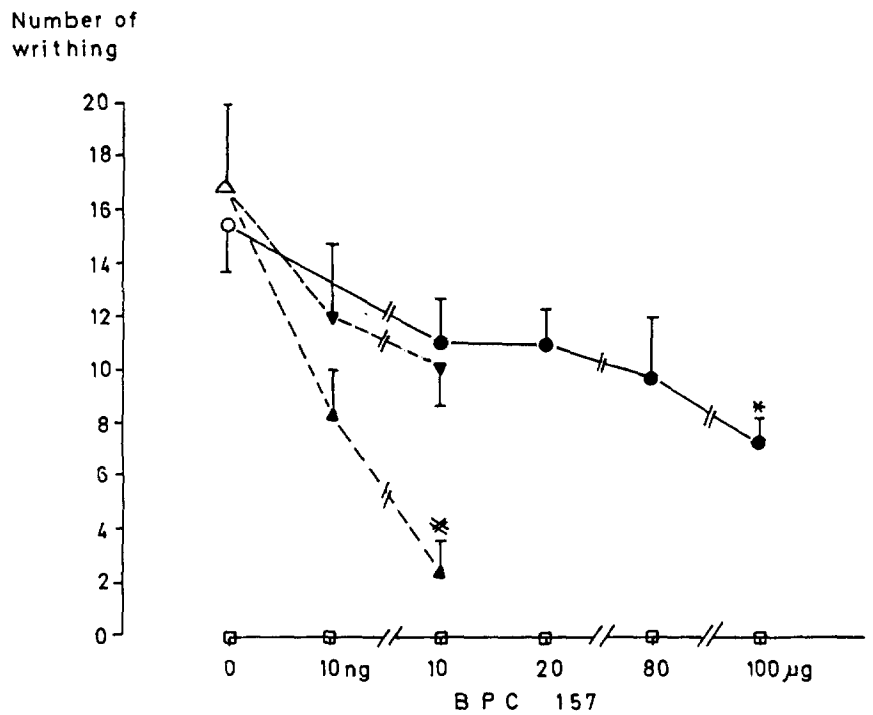

Figure 1. Writhing (acetic acid/magnesium sulphate) assay. BPC 157 (●▲▼ □) (dose/kg b.w.) or 0.9% NaCl (5.0 ml/kg b.w.) were applied i.p. in writhing procedures: (i) acetic acid (○●), 30 min before the challenge (●); (ii) magnesium sulphate (△▲▼), simultaneously (▲) or 30 min before (▼) the challenge; vs. control $*p<0.05$, $\#p<0.01$. BPC 157 application does not induce by itself (□) any writhing movement. Means ± SEM. $n=16–20$ mice per each experimental group

DISCUSSION

The involvement of BPC in nociception as shown by these results, seems to be in line with the behaviour of a number of peptides known to be present in the different pathways subserving pain [17,18]. The possible analgesic mechanism of the peptide appears to be complex.

This seems to be supported by the evidence that BPC 157 reduced the nociceptive response in various assays, involving apparently different pathways, including both indirect as well as direct pain stimulation [6–17], such as that seen with acetic acid (liberation of one or more substances exciting nociceptors) and magnesium sulphate (direct stimulation of peritoneal nociceptors) [8–11]. The acetic acid assay, as a model of inflammatory, PG-dependent pain, has been connected both with acute inflammation of the peritoneal area [8] and with increased levels of PGE_2. $PGF_{2\alpha}$ [19,20] as well as malondialdehyde (MDA), a metabolite formed during PG synthesis from endoperoxides in peritoneal fluid [9,10]. While the magnesium sulphate assay is considered to be a model of non-inflammatory and non-prostaglandin-dependent pain, since there are no changes in either PG release in the peritoneum, or in MDA

concentration in the peritoneal fluid [9,10]. Consistent with those differences, the NSAIDs, serotonin and histamine-receptor-blocking drugs and morphine (known to be effective in acetic acid assay) are active only in much higher dosages, if at all, in the magnesium sulphate assay [9,10]. Interestingly, BPC 157 seems to be more effective in the magnesium sulphate than in the acetic acid assay. Hence, besides a modulation of the pain-producing effect of PGs and other substances, BPC 157 also appears to have an immediate (since it is effective when applied simultaneously with magnesium sulphate) direct effect on peritoneal pain endings, which is likely to be unrelated to PGs [8–11].

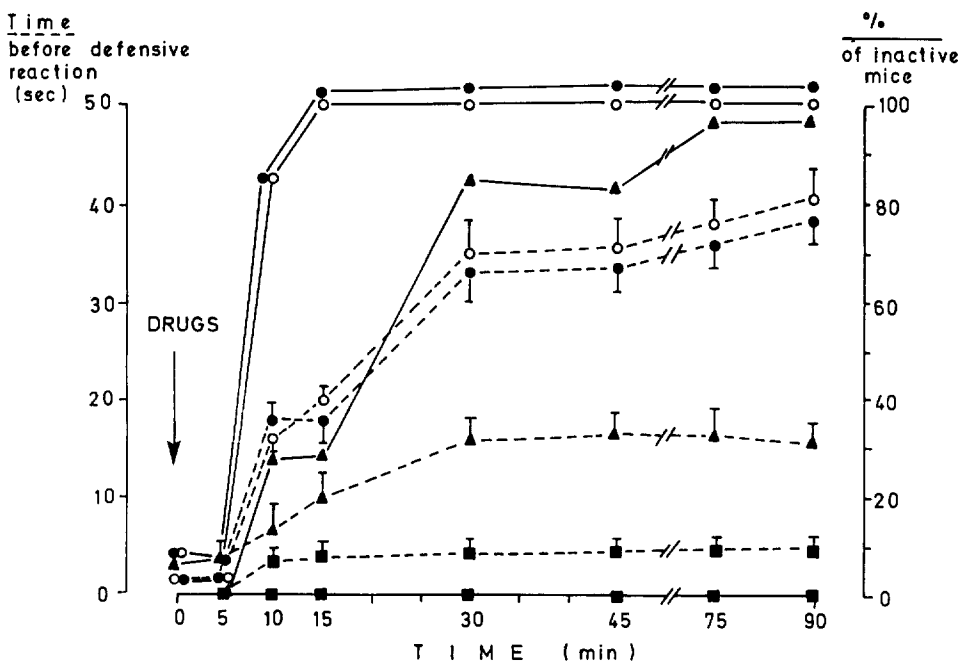

Figure 2. Tail-pinching test. Treatment (dose/kg b.w. i.p.) BPC 157 10 μg (○), 10 ng (●), aspirin 100 mg (▲), 0.9% NaCl 5 ml (■). Time before defensive reaction (-----) (vs. control: $p<0.001$ (10–90 min (BPC 157), 15–90 min (aspirin); BPC 157 vs. aspirin $p<0.05$ (10–90 min); % of inactive mice (——) ($p<0.001$: BPC 157, aspirin vs. control (10–90 min); BPC 157 vs. aspirin (10–15 min)). Means ± SEM. $n=16$–20 mice per each experimental group

Consequently, it seems that the BPC 157 analgesic effect could be different from that of NSAIDs or morphine. This seems to be supported by the evidence that BPC 157 is more active than aspirin in, e.g. the tail pinching test. Likewise, in contrast to morphine, BPC 157 was not effective in the hot-plate test on naive mice or rats. Finally, the influence of BPC 157 on pain modulation, in line with its strong anti-inflammatory effect, should be viewed in conjunction with its proposed organoprotective effects [1–4], particularly a prominent gastrointestinal protection and a beneficial effect in wound healing and haemorrhagic shock (unlike the deleterious effect of NSAIDs or endogenous opioides [4,21–23].

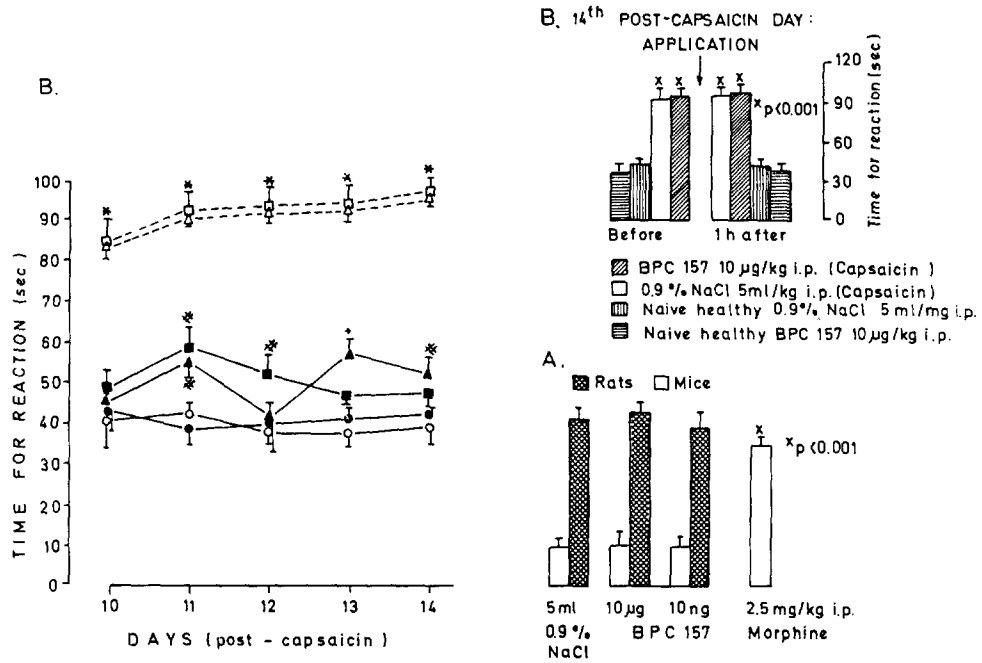

Figure 3. Hot plate assay. (A) The effects in normal animals. (B) The effects in normal rats (daily drugs application/kg, b.w. i.p. 1 h before hot plate procedure: ○: 0.9% NaCl 5.0 ml, ●: BPC 157 10 μg) vs. capsaicin (day 0) (125 mg/kg b.w. s.c.) treated groups (daily drugs application/kg, b.w. i.p. 1 h before hot plate procedure: □: 0.9% NaCl 5.0 ml, ■: BPC 157 10 μg; single application/kg b.w. i.p. 1 h before capsaicin: △: 0.9% NaCl 5.0 ml, ▲:BPC 157 10 μg) rats. Days (10–14) post-capsaicin are shown. Means ± SEM, Capsaicin-saline (□△) vs. BPC 157-capsaicin (■▲) and corresponding normal rats (○●) *$p<0.001$; BPC 157-capsaicin (■▲) vs. corresponding normal rats (○●) #$p<0.05$, +$p<0.001$. $n=16$–20 animals per each experimental group

The peptide's specific activity in nociception seems to be supported by its influence on allodynia in animals treated with a neurotoxic dose of capsaicin [17] and later subjected to the hot-plate test. Since, in this assay, BPC 157 had no influence on the normal pain reaction in naive animals, and, when given as a single pretreatment injection, it protected capsaicin-treated animals from neurotoxicity, a close interaction with capsaicin seems likely. Furthermore, the development of capsaicin neurotoxicity was prevented by daily injection of BPC 157 while it had no influence after capsaicin degeneration had been established. Thus, it is possible that BPC 157 activity is related to the integrity of capsaicin-sensitive somatosensory neurons and their protection. Therefore, primary afferent neurons having small-diameter somata and unmyelinated (C–) or thinly myelinated (Aδ–) fibres (as a main target of the sensory neuron-selective effects of capsaicin), and intracellular accumulation of calcium and NaCl, either slowly reversible or irreversible (with or without degeneration of neuronal soma), associated with quick defunctionalization and delayed depletion of

cellular constituents and peptide transmitters (as final events implicated in the mechanism of the capsaicin neurotoxicity) [17], could be involved in BPC's action in nociception.

Those qualities, together with efficacy after oral application and no or negligible toxicity (100 mg/kg had no untoward effect in an acute toxicology study [4]), make BPC 157 an interesting tool for further research.

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