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GASTRIC PENTADECAPEPTIDE BPC 157 COUNTERACTS MORPHINE-INDUCED ANALGESIA IN MICE

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Previously, the gastric pentadecapeptide BPC 157, (PL 14736, Pliva) has been shown to have several beneficial effects, it exert gastroprotective, anti-inflammatory actions, stimulates would healing and has therapeutic value in inflammatory bowel disease. The present study aimed to study the effect of naloxone and BPC 157 on morphine-induced antinociceptive action in hot plate test in the mouse. It was found that naloxone and BPC 157 counteracted the morphine (16 mg/kg s.c.) - analgesia. Naloxone (10 mg/kg s.c.) immediately antagonised the analgesic action and the reaction time returned to the basic values, the development of BPC 157-induced action (10 pg/kg, 10 ng/kg, 10 μg/kg i.p.) required 30 minutes. When haloperidol, a central dopamine-antagonist (1 mg/kg i.p.), enhanced morphine-analgesia, BPC 157 counteracted this enhancement and naloxone reestablished the basic values of pain reaction. BPC 157, naloxone, and haloperidol per se failed to exert analgesic action. In summary, interaction between dopamine-opioid systems was demonstrated in analgesia, BPC 157 counteracted the haloperidol-induced enhancement of the antinociceptive action of morphine, indicating that BPC acts mainly through the central dopaminergic system.

K e y w o r d s: *gastric pentadecapeptide BPC 157, dopamine, naloxone, haloperidol, morphine, antagonization, analgesia*

INTRODUCTION

Besides the activation of the opioid system, morphine-analgesia encompasses many other neurotransmitter systems, and different peptides (1-5). Particularly interesting may be gut peptides (6-8). Thereby, the present study aims to focus on morphine analgesia and gastric pentadecapeptide BPC 157 (GEPPPGKPADDAGALV, M.W. 1419). It is an anti-ulcer peptide (9-12), interacting with NO-system (13), and, unlike other peptides, it is particularly stable in gastric juice (14) and is used consistently without a carrier (9-11, 13-24), being safe in patients with the inflammatory bowel disease (coded PL-10, PLD 116, PL 14736) (21).

Noteworthy, given peripherally, this peptide counteracts several central nervous system disturbances. Firstly, during its either acute or chronic administration, highly specific alpha-methyl-L-tryptophan autoradiography measurements reveal that it affects the central nervous system neurotransmitters synthesis and function in specific regions. An increased serotonin synthesis in rat substantia nigra´s (compacta and reticulate) structure (11, 16, 19, 22-25) parallels the antidepressant effect during the Porsolt's test (17). BPC 157 reduces the duration of immobility to a greater extent than imipramine does (17) and, in addition, it counteracts the serotonin syndrome in rats (23). Secondly, it may prevent and reverse catalepsy or stereotypy due to the central dopamine system failure (11, 19). Interestingly, it also counteracts behavioral disturbances in amphetamine treated rats, acutely and chronically, and the haloperidol-amphetamine -

climbing behavior (18, 19). Thirdly, gastric pentadecapeptide BPC 157 counteracts and reverses the behavioral disturbance induced by both acute and chronic alcohol ingestion (22, 24). Furthermore, it counteracts also gastric and liver lesions, induced by acute or chronic administration of alcohol (13, 20).

Thus, this study has shown that pentadecapeptide BPC 157 counteracts morphine-induced analgesia. Besides the morphine-antagonist naloxone, the central dopamine antagonist haloperidol has also been tested, presenting this peptide's close interaction with the dopamine system (11, 15-17, 19).

MATERIAL AND METHODS

Animals

Male Hannover NMRI mice 25-30 g b.w. randomly selected were used in all of the experiments. The mice were housed in cages placed in a quiet and temperature-humidity controlled room (22°C±1-2°C) with a 12 hr light-dark cycle. Food and water were available *ad libitum*. All of the experiments were approved by the local ethics committee.

Drugs

Pentadecapeptide BPC 157 (GEPPPGKPADDAGLV, MW 1419 Da; manufactured by Diagen, d.o.o., Ljubljana, Slovenia)

(9-11, 13-24), morphine Merck 20 (Merck KGaA, Darmstadt, Germany), naloxone hydrochloride (Sigma-Aldrich, Germany), haloperidol (Sigma-Aldrich, Germany) were used. All drugs were dissolved in saline, and freshly prepared before starting the experiment.

Assessment and medication application

The analgesia test was performed on the mice receiving morphine prior to the tested agents (i), and on the mice receiving no morphine prior to tested agents (ii), using the hot plate (Ugo Basille, Italy). The hot plate was maintained at the temperature of 55°C, and experimental protocol as previously described (1, 26). The reaction time was defined by placing the mouse on the hot plate, and the time was recorded when the animal showed hind paw licking or jumping, and the 45 sec period was observed to avoid tissue damage. Before any experimental treatment was administered (time "-"), each mouse was initially tested with the hot-plate test to determine its basal reaction time-period.

The medication (morphine (16 mg/kg s.c.), the tested agents (gastric pentadecapeptide BPC 157 (10 pg, 10 ng and 10 μg/kg i.p.), naloxone (10 mg/kg s.c.), haloperidol (1 mg/kg i.p.) or saline (5 ml/kg i.p.) was given accordingly at either 30 min, 20 min or 10 min before the first assessment (time "0"), and subsequently, the mice were assessed at 15, 30, 45, 60, 90, 120, 150 and 180 min intervals (time "+").

Specifically, to assess basal analgesia, (i) the first assessment (time "0") was preceded by the application of morphine or saline 30 min before (time "-30 min"), then by the application of the gastric pentadecapeptide BPC 157 or naloxone or saline 20 min before (time "-20 min"), and finally, by the application of the saline 10 min before (time "-10 min"). To assess enhanced analgesia, the first assessment at time "0" was preceded by the application of morphine or saline 30 min before (time "-30 min"), by the application of haloperidol or saline 20 min (time "-20 min"), and, lastly, by the application of gastric pentadecapeptide BPC 157 or naloxone or saline (5 ml/kg i.p.) 10 min (time "-10 min").

On the other hand, (ii) the first assessment of the analgesia in the absence of morphine (time "0"), the application of saline was administered 30 min in advance (time "-30 min"), the application of haloperidol or gastric pentadecapeptide BPC 157 or naloxone or saline 20 min in advance (time "-20 min"), and the application of saline 10 min in advance (time "-10 min").

Statistical analysis

Statistical analysis was done with parametric analysis variance (one-way ANOVA test). If P was equal or less than 0.05 (P≤0.05), the subsequent post hoc analysis with the Tukey HSD test was performed. The results are graphically presented as the mean±S.E.M.
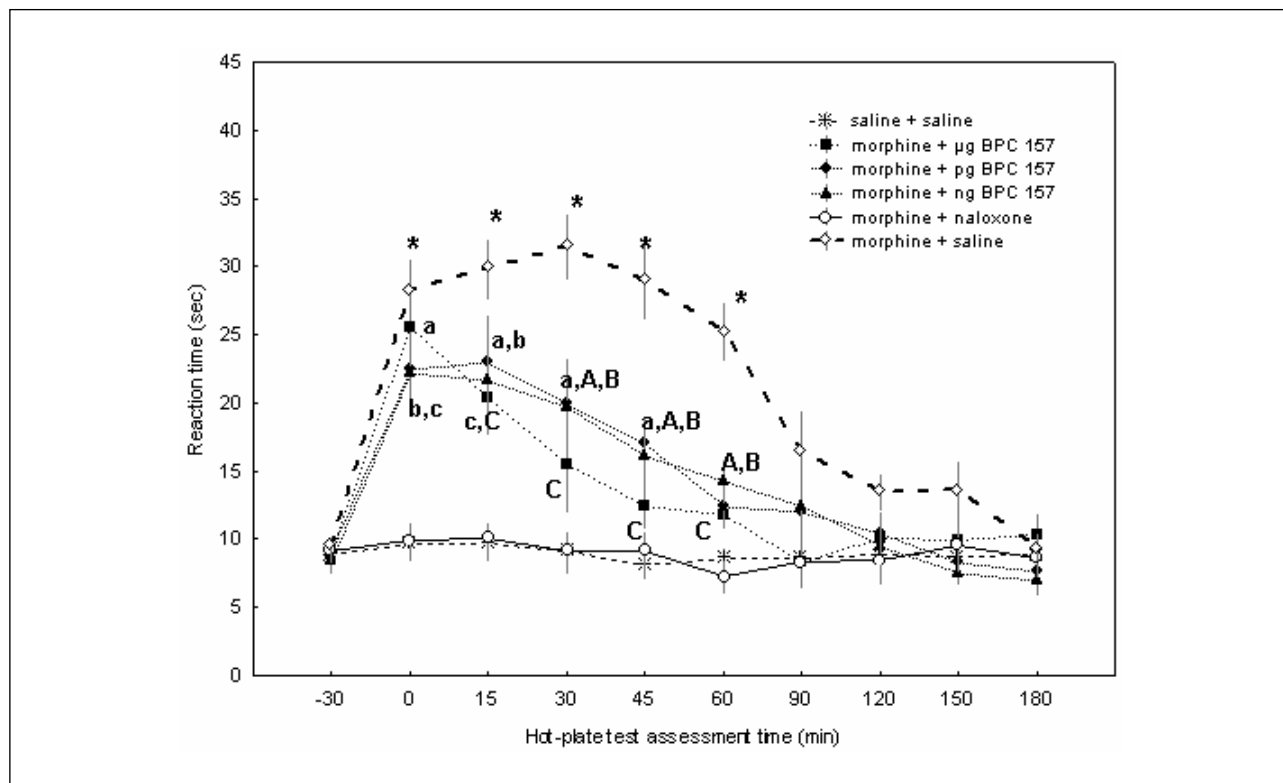

Fig. 1. Analgesia assessment, the first assessment (time "0") was preceded at 30 min before (time "-30 min") by the application of the morphine (16 mg/kg s.c.) or saline (5 ml/kg i.p.), at 20 min before (time "-20 min") by the application of the gastric pentadecapeptide BPC 157 (10 pg, 10 ng and 10 μg /kg i.p.) or naloxone (10 mg/kg) or saline (5 ml/kg i.p.), and finally, at 10 min before (time "-10 min") by the application of the saline (5 ml/kg i.p.). In naive mice the pentadecapeptide BPC 157 or naloxone administrated alone produce no effect on reaction time of the mice placed on a hot plate (data not specifically shown). Ten or twelve mice were used in each experimental group and each mouse was used only once. One-way Anova test and post hoc analysis with the Tukey HSD test was performed. P≤ 0.05 *vs.* saline (saline + saline + saline): * - morphine (morphine + saline + saline), (a) - morphine + pg BPC 157 + saline, (b) - morphine + ng BPC 157 + saline, (c) - morphine + μg BPC 157 + saline. P≤0.05 *vs.* morphine (morphine + saline + saline): (A) - morphine + pg BPC 157 + saline, (B) - morphine + ng BPC 157 + saline, (C) - morphine + μg BPC 157 + saline.
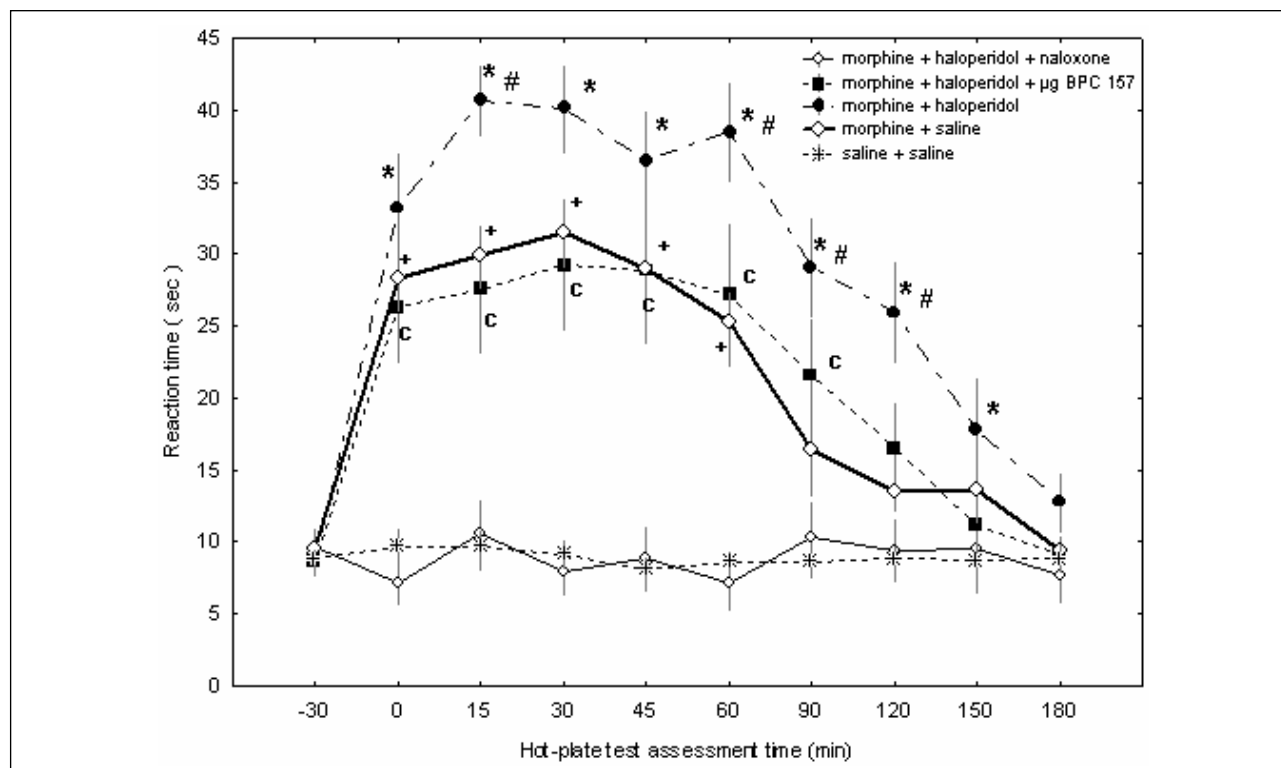
Fig. 2. Assessment of enhanced analgesia, with the first assessment as time "0", the application of the morphine (16 mg/kg s.c.) or saline (5 ml/kg i.p.) was at 30 min before (time "-30 min"), the application of the haloperidol (1 mg/kg i.p.) or saline (5 ml/kg i.p.) at 20 min before (time "-20 min"), and at 10 min before (time "-10 min") the application of the gastric pentadecapeptide BPC 157 (10 pg, 10 ng and 10 μg /kg i.p.) or naloxone (10 mg/kg) or saline (5 ml/kg i.p.). In naive mice the pentadecapeptide BPC 157, naloxone or haloperidol administrated alone produce no effect on reaction time of the mice placed on a hot plate (data not specifically shown). Ten or twelve mice were used in each experimental group and each mouse was used only once. One-way Anova test and post hoc analysis with the Tukey HSD test was performed. $P \leq 0.05$ *vs.* saline (saline + saline + saline): * - morphine + haloperidol + saline, + - morphine + saline + saline, © - morphine + haloperidol + μg BPC 157. $P \leq 0.05$ *vs.* morphine + saline + saline: # - morphine + haloperidol + saline.

RESULTS

In the saline treated mice, pentadecapeptide BPC 157, naloxone or haloperidol administrated alone produce no effect on the reaction time of the mice placed on a hot plate (the data not specifically shown). Morphine consistently induces long-lasting analgesia (*i.e.*, the reaction time exceeds the values shown in naive saline treated mice). This effect is counteracted by either pentadecapeptide BPC 157 or naloxone. Given after morphine, they both decrease reaction time to the values observed in the control saline mice (*Fig. 1*).

On the other hand, haloperidol given after morphine prolongs the reaction time compared to the morphine group. In these morphine + haloperidol treated mice, BPC 157 or naloxone, both reverse the overly increased values. Pentadecapeptide BPC 157 restores the values obtained for the morphine-analgesia itself. At the same time, naloxone restores the values seen in the saline treated mice (*Fig. 2*).

DISCUSSION

Both naloxone, a morphine antagonist, and gastric pentadecapeptide BPC 157 promptly counteract the morphine-analgesia effect in the hot-plate tested mice. Since these results are obtained repeatedly, with different doses of gastric pentadecapeptide BPC 157, they are obviously not random. In relation to the values for the saline treated mice, naloxone

reacted immediately, while BPC 157 required 30 minutes' period. When a central dopamine-antagonist haloperidol enhances morphine-analgesia, BPC 157 counteracts this aggravation, naloxone reestablishes the healthy values. This amplification of opioid analgesia goes along with the suggested role of the dopamine system failure, and the tonically active anti-opioid system (24-27). Thereby, this point discriminates the observed naloxone counteraction of morphine-analgesia from that achieved with pentadecapeptide BPC 157. Thus, it could be that in morphine analgesia resulting from the dopamine-opioid systems' interaction, the BPC 157's anti-morphine activity mainly concerns the central dopamine system. Noteworthy, without morphine-analgesia, BPC 157, naloxone, and haloperidol are found to be ineffective.

Previously, it was shown that pentadecapeptide BPC 157-dopamine system interaction is quite complex. This peptide counteracts both the haloperidol-catalepsy (11) and the amphetamine-stereotypies (acute and chronic) (18, 19). Also, it counteracts the climbing behavior that results from a particularly combined administration of haloperidol and the subsequent amphetamine regimen (19). The therapeutic effect of BPC 157 on gastric mucosa during stress or other ulcerogenic procedures was ascribed to the dopaminergic system in the brain (12, 15). Noteworthy, when used in the same dose-range, BPC 157 counteracts all of the concomitant gastrointestinal lesions, induced by haloperidol, vesicles depletor reserpine, or neurotoxin affecting substantia nigra 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropropyridine (MPTP) (10, 11, 16).

These effects should be combined with the BPC 157 ability to counteract alcohol intoxication, both acute and chronic (22, 24) (commonly believed to be relevant to the disturbances induced by morphine application (5). Along with its counteraction of serotonin syndrome (23), as well as Porsolt´s test-helpless behaviour (17), highly specific alpha-methyl-L-tryptophan autoradiographic measurements reveal that it affects central nervous system neurotransmitters synthesis and function in brain specific regions when it was given peripherally (25). Substantia nigra's (compacta and reticulata) structure is thought to be the most important one for the increased serotonin synthesis induced by the BPC 157 acute and chronic peripheral application (25). Also this structure is related to the dopamine and serotonin interaction with the opioidergic system (31, 32).

In summary, being safe in patients with the inflammatory bowel disease, and devoid of toxic effects (LD1 could be not achieved, limit test negative) (21), gastric pentadecapeptide BPC 157 consistently counteracts analgesia in the mice placed on a hot plate and treated with morphine or morphine + haloperidol. Further studies should additionally investigate whether these anti-morphine effects indeed reflect the complex interaction of the dopamine and serotonin systems (33) with the opioidergic system.

Conflict of interests: None declared.

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