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Gastric pentadecapeptide BPC 157 effective against serotonin syndrome in rats

Alenka Boban Blagaic, Vladimir Blagaic, Mirela Mirt, Nikola Jelovac, Goran Dodig, Rudolf Rucman, Marijan Petek, Branko Turkovic, Tomislav Anic, Miroslav Dubovecak, Mario Staresinic, Sven Seiwerth, Predrag Sikiric*

Department of Pharmacology, Medical Faculty, University of Zagreb, Salata 11, POB 916, 10000 Zagreb, Croatia

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Abstract

Serotonin syndrome commonly follows irreversible monoamine oxidase (MAO)-inhibition and subsequent serotonin (5-HT) substrate (in rats with fore paw treading, hind limbs abduction, wet dog shake, hypothermia followed by hyperthermia). A stable gastric pentadecapeptide BPC 157 with very safe profile (inflammatory bowel disease clinical phase II, PL-10, PLD-116, PL-14736, Pliva) reduced the duration of immobility to a greater extent than imipramine, and, given peripherally, has region specific influence on brain 5-HT synthesis (α-[^{14}C]methyl-L-tryptophan autoradiographic measurements) in rats, different from any other serotonergic drug. Thereby, we investigate this peptide (10 μg, 10 ng, 10 pg/kg i.p.) in (i) full serotonin syndrome in rat combining pargyline (irreversible MAO-inhibition; 75 mg/kg i.p.) and subsequent L-tryptophan (5-HT precursor; 100 mg/kg i.p.; BPC 157 as a co-treatment), or (ii, iii) using pargyline or L-tryptophan given separately, as a serotonin-substrate with (ii) pargyline (BPC 157 as a 15-min posttreatment) or as a potential serotonin syndrome inductor with (iii) L-tryptophan (BPC 157 as a 15 min-pretreatment). In all experiments, gastric pentadecapeptide BPC 157 contrasts with serotonin-syndrome either (i) presentation (i.e., particularly counteracted) or (ii) initiation (i.e., neither a serotonin substrate (counteraction of pargyline), nor an inductor for serotonin syndrome (no influence on L-tryptophan challenge)). Indicatively, severe serotonin syndrome in pargyline+L-tryptophan rats is considerably inhibited even by lower pentadecapeptide BPC 157 doses regimens (particularly disturbances such as hyperthermia and wet dog shake thought to be related to stimulation of 5-HT$_{2A}$ receptors), while the highest pentadecapeptide dose counteracts mild disturbances present in pargyline rats (mild hypothermia, feeble hind limbs abduction). Thereby, in severe serotonin syndrome, gastric pentadecapeptide BPC 157 (alone, no behavioral or temperature effect) has a beneficial activity, which is likely, particular, and mostly related to a rather specific counteraction of 5-HT$_{2A}$ receptors phenomena.

Keywords: Gastric pentadecapeptide BPC 157; Pargyline/L-tryptophan; Serotonin syndrome; 5-HT$_{2A}$ receptor antagonism; (Rats)

1. Introduction

This study focus serotonin (5-HT) syndrome, the most serious toxic interaction of antidepressants (i.e., Houlihan, 2004) and attempts to further clarify an intriguing effect of a peripheral peptide, gastric pentadecapeptide BPC 157, on brain 5-HT synthesis and release (Tohyama et al., 2004). Namely, it initially reduced the duration of immobility to a greater extent than imipramine, suggestive for involvement of the brain serotonergic and noradrenergic systems (Sikiric et al., 2000). Currently, gastric pentadecapeptide BPC 157 (GEPPPGKPADDAGLV, M.W.1419; i.e., Sikiric et al., 1994, 1997a,b, 1999, 2003; Jelovac et al., 1999; Bilic et al., 2001; Lovric-Bencic et al., 2004; Tohyama et al., 2004; Veljaca et al., 2002) is promising in inflammatory bowel disease clinical phase II (PL-10, PLD-116, PL-14736, Pliva). More importantly, besides reduced duration of

* Corresponding author. Tel.: +385 1 4566 833, +385 1 4566 834; fax: +385 1 492 00 50.
E-mail address: sikiric@mef.hr (P. Sikiric).

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immobility and particular effect on brain 5-HT synthesis (Tohyama et al., 2004; Sikiric et al., 2000), also given peripherally, it affects various disturbances supposed to be centrally mediated (Jelovac et al., 1998, 1999; Sikiric et al., 1999, 2001, 2002; Boban Blagaic et al., 2004)) by the systems (i.e., dopaminergic, GABA-ergic) (Nisijima et al., 2001, 2003) involved also in 5-HT disturbances.

As indicated, very recently, using α-[^{14}C]methyl-L-tryptophan autoradiographic measurements, gastric pentadecapeptide BPC 157, a gut peptide, given peripherally, has region specific influences on brain 5-HT synthesis in rats, different following acute and chronic treatments (Tohyama et al., 2004). The effects of BPC 157 reported (Tohyama et al., 2004) do not resemble the results obtained with any other serotonergic drug using this method (Diksic, 2001; Diksic and Young, 2001; Diksic et al., 1995; Tohyama et al., 2002). This may suggest that although BPC 157 has an effect on 5-HT synthesis in some brain structures in which 5-HT synthesis is affected by drugs acting on the brain serotonergic system (e.g., fluoxetine, paroxetine), the mechanism of the BPC 157 action probably differs greatly (Tohyama et al., 2004). Most likely, this peptide has a prompt and inherent action since it also reduces antidepressant-arrhythmias, and severe cardiotoxicity (Lovric et al., 2003; Lovric-Bencic et al., 2004). Its particular stability (i.e., no degradation in otherwise highly degradating condition (i.e., Sikiric et al., 1994, 1997a,b, 1999, 2003; Jelovac et al., 1999; Lovric et al., 2003; Lovric-Bencic et al., 2004; Boban Blagaic et al., 2004), non-degraded in human gastric juice (Veljaca et al., 1995), and non-toxicity (even limit test negative, no side effects in trials (Veljaca et al., 2002)) certainly both contribute. Together, these findings could be of both theoretical and practical importance for antidepressants therapy.

Thus, in present study, presenting with a beneficial effect mostly related to a counteraction of 5-HT$_{2A}$-receptor phenomena, pentadecapeptide BPC 157 (in doses previously used (Sikiric et al., 1993; Sikiric et al., 1994, 1997a,b, 1999, 2000, 2002, 2003; Jelovac et al., 1998, 1999; Lovric et al., 2003; Lovric-Bencic et al., 2004; Tohyama et al., 2004; Boban Blagaic et al., 2004) particularly counteracts a severe 5-HT syndrome otherwise induced with pargyline (irreversible monoamine oxidase (MAO)-inhibition)/L-tryptophan (5-HT precursor) application (i.e., rats presented with fore paw treading, hind limbs abduction, wet dog shake, hypothermia followed by hyperthermia).

2. Materials and methods

2.1. Animals

Male Wistar rats aged 35 days weighing 150 g were used in all of the experiments accordingly with study of Darmani and Ahmad (1999), randomly assigned. The rats where housed at a room temperature of 22 °C ± 1–2 °C with a 12-h light–dark cycle singly caged, for 14 days before the experiment. Food and water were available ad libitum. Local ethic committee approved all of the experiments.

2.2. Drugs

Pargyline HCl (Sigma, USA) and pentadecapeptide BPC 157 (GEPPPGKPADDAGLV, molecular weight 1419 Da, manufactured by Diagen, Slovenia; Sikiric et al., 1993; Sikiric et al., 1994, 1997a,b, 1999, 2000, 2002, 2003; Jelovac et al., 1998, 1999; Lovric et al., 2003; Lovric-Bencic et al., 2004; Tohyama et al., 2004; Boban Blagaic et al., 2004) were dissolved in saline; L-tryptophan (Merck, Germany) was suspended in saline containing 0.5% carboxymethil cellulose sodium (Abdel-Fattah et al., 1995, 1996, 1997). All drugs were freshly prepared before starting the experiments.

2.3. Experimental protocol

The agents, pargyline (75 mg/kg), L-tryptophan (100 mg/kg), gastric pentadecapeptide BPC 157 (10 μg, 10 ng, 10 pg/kg), or saline (5.0 ml/kg), were given intraperitoneally (i.p.; Sikiric et al., 1993; Sikiric et al., 1994, 1997a,b, 1999, 2000, 2002, 2003; Jelovac et al., 1998, 1999; Lovric et al., 2003; Lovric-Bencic et al., 2004; Tohyama et al., 2004; Boban Blagaic et al., 2004; Abdel-Fattah et al., 1995, 1996, 1997). As described, 5-HT syndrome was induced with pargyline (75 mg/kg i.p.) and subsequent L-tryptophan (100 mg/kg i.p.) application, given at 15 min thereafter. Pentadecapeptide BPC 157 was challenged alone, in rats treated with either L-tryptophan or pargyline, and finally in pargyline-pretreated rats challenged with L-tryptophan as follows: (i) To discriminate whether pentadecapeptide BPC 157 could provoke a temperature change or even a 5-HT syndrome, pentadecapeptide BPC 157 (or an equivolume of saline) was given at 15 min before L-tryptophan (or an equivolume of saline). To point out the possible influence on MAO-inhibition induced by pargyline, or whether pentadecapeptide BPC 157 could be a 5-HT-substrate as L-tryptophan, pentadecapeptide BPC 157 (or an equivolume of saline) was given at 15 min after pargyline. (ii) Considering the influence of pentadecapeptide BPC 157 on 5-HT syndrome presentation in pargyline pretreated rats challenged with L-tryptophan, pargyline was applied at 15 minutes before L-tryptophan co-administered with pentadecapeptide BPC 157 or saline.

2.4. Assessment

The procedure (Darmani and Ahmad, 1999) was generally adopted. The behavior assessment was just before first administration and every 15 min to 90 min, and thereafter, at 150 min, and at 210 min for 15 sec (fore paw treading, hind limbs abduction) or 5 min (wet dog shake). The behavioral disturbance severity was assessed as a score (0–3) of fore paw treading or hind limbs abduction (score 0—absent,