Hacettepe University, Journal of the Faculty of Pharmacy
Volume 24 / Number 2 / July 2004 / pp. 57-64

Synthesis and Investigation of Antinociceptive and Antidepressant Effects of the Stereoisomers of a New Dipeptoid Analog of Cholecystokinin

Received : 18.11.2004
Revised : 27.01.2005
Accepted : 10.02.2005

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Introduction

Cholecystokinin (CCK) is a peptide hormon that functions as a gastrointestinal system regulator in the periphery and as a neuro transmitter in the central nervous system (CNS) by way of two receptor subtypes designated as CCK-A (in the periphery) and CCK-B (in CNS). The results of the studies aimed to reveal the physiological roles of CCK in the CNS have shown that CCK-B receptors play a role in analgesia and depression as well as anxiety, learning and memory and panic disorder [1]. A number of peptide, non-peptide and peptidomimetic CCK-B receptor antagonists and agonists have been prepared as potential therapeutic agents and useful tools for studying the physiological and pharmacological roles of CCK[2]. Amongst these agents dipeptoids with high affinity and selectivity to CCK receptors have been developed starting from the chemical lead Boc-Trp-Phe-NH_2 (M0) by rational drug design based on in vitro receptor binding studies[3-8]. Additionally several researchers have reported naloxone- reversible antinociceptive and antidepressant effects of CCK_8 or its analogues and a functional antagonism between CCKergic and opiodergic systems have been evidenced[9,10].

Following these findings many studies have been focused on the potentiation of the opiate produced antinociception by CCK-B antagonists where the interactions between CCK receptors and the opioid system via μ -opioid (antinociceptive effect) and δ-opioid (antidepressant effect)

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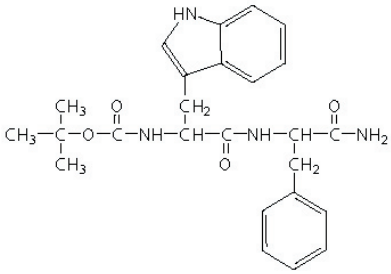
receptors have also been investigated[11-14]. Recently design and synthesis of new dipeptide mimetics having dual interaction ability with both cholecystokinin and opioid receptors have been reported[15-17].

Despite the ongoing studies on the stereochemical aspects of the interaction of CCK with its analogs[6-8], the role of the Phe-NH_2 group and the stereochemistry of those effects have not been clarified.

In this study we aimed to investigate the antinociceptive and antidepressant effects of Boc-Trp-Phe-NH_2 in comparison with stereoisomers of its analog modified at the C-terminal by morpholine moiety which brings about sterical effect and adds an additional hydrogen binding site to the molecule.



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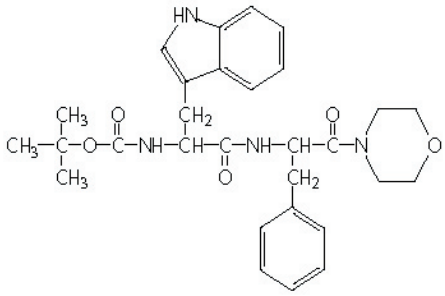

M1-M4

Materials and Methods

Chemistry

Melting points were determined by using Thomas Hoover Capillary Melting Point Apparatus and are uncorrected (Philadelphia, PA, USA). Infrared spectra was obtained from Perkin-Elmer 1720 X FT-infrared (Beaconsfield, UK). ^{1}H-NMR Spectra were recorded by Bruker DPX-400, 400 MHz High Performance Digital FT -NMR (BioSpin Siberstrifen 4, 76287 Rheinstetten Germany). Optical rotations were measured with a Rudolph Research Analytical polarimeter (New Jersey, Flanders, USA). Mass spectrometric analysis were carried out with HP1100LC/MS (ESI). TLC was performed on Silicagel (Kieselgel 60 F 254 Merck). For column chromatography silica gel, for gel filtration Sephadex LH-20 (Pharmacia) were used.

General procedures for the synthesis of the compounds, M1-M4:

Protection of amino acids

Protection of the carboxyl group of phenylalanine and amino group of tryptophan were performed according to the published procedures [18]. L-Phe-NH_2 and D-BocTrp were obtained from commercial sources (Sigma Chemical Comp.).

L-Phe-OMe.HCL yield=86%, m.p= 154-155°C, Lit [19] m.p=155°C

D- Phe-OMe:HCl yield= 46%, m.p=161.1 °C, Lit [20] m.p=154-159° C

L -BocTrp yield= 38.75%, m.p= 138-140 °C, Lit [21] m.p.=136.5-140.5°C

Coupling Reactions

Comps. M1-M4

Couplings were performed by the DCC- HOBt method in DMF at room temperature for 24-36 h[22]. The residues obtained after evaporation of the solvent in vacuo, were dissolved in EtOAc and was washed successively with 1N HCl or 5% citric acid; 5 % $NaHCO_3$ and saturated NaCl, dried over Na_2SO_4 and concentrated. The crude product was purified by column chromatography (Silicagel, AcOEt/n-Hexane)

Comp.M0 was obtained by mixed anhydride method according to the literature[3].

Saponification of the dipeptide Boc-Trp-Phe-OMe:

The peptide was hydrolyzed with 1 N NaOH (2-3 eq) in MeOH below 25° C. After the saponification was completed, the solution was neutralized with 1N HCl and evaporated to remove MeOH. The residual solution was acidified to pH 3 and extracted with EtOAc. The extract was washed with saturated NaCl, dried over Na_2SO_4 and concentrated. The product Boc-Trp-Phe-COOH was used for the next step without further purification.

Boc-Trp-Phe-CO-morpholidide:

^{1}H- NMR ($CDCl_3$) δ : 1.4-1.5 (s, 9H), 2.6- 3.2 (m, 6H), 3.2-3.5 (m, 6H), 4.4(s,1H), 4.8-5.1 (m, 2H), 6.5-7.6 (m,10H aromatic Hs), 7.6-8.1 (s,1H).

MS m/z 543 (22%,M+Na^+), 521 (100%, M+2)

MS/MS m/z, 421.1(10.2%) , 334.1 (100%) , 306.2 (92.5%100), 289.1 (10.4%) , 235.1 (5%), 185.0 (2%)

M1 (RS) Boc-Trp-Phe-CO-morpholidide: 45.78 % yield ; mp 153-155°C ; $[\propto]_D^{20}$ (C=0.05, MeOH) : - 7.997

M2 (SR) Boc-Trp-Phe-CO-morpholidide : mp 155°C ; $[\propto]_D^{20}$ (C=0.05, MeOH): + 7.997

M3 (RR) Boc-Trp-Phe-CO-morpholidide: mp 130°C ; $[\propto]_D^{20}$ (C=0.05, MeOH): + 3.999

M4 (SS) Boc-Trp-Phe-CO-morpholidide:mp 129-130°C; $[\alpha]_D^{20}$ (C=0.05, MeOH) : - 3.999

M0 (R,S)-Boc-Trp-Phe-NH2: mp 149-151 °C .

^{1}H- NMR ($CDCl_3$) δ : 1.3-1.5 (s, 9H), 3.0-3.3 (m, 4H), 4.18 (m,1 H), 4.64 (q,1H), 5.0-5.2 (d, 2H), 5.9 (m,1H), 6.4-7.6 (m,10 H aromatic), 8.0-8.1(s,1H)

Pharmacology

Albino mice weighing 20-25 g were used in the present study. Laboratory temperature was maintained at 20 °C under conditions of a 12 hour light dark schedule. Before experimentation, mice were allowed 1 week of adaptation. They were used only once. The study was approved by Committee of Ethic at Osmangazi University Medical School.The animals were divided into 9 groups, 8 animals each.

All the compounds were dissolved in DMSO and given to the animals intraperitoneally at 5 mg/kg doses. 0.1 mg/ kg naloxone administration was performed i.p. 15 min after the injection of the compounds. Control animals received i.p. 0.1 ml DMSO.

Tail Clip Test, tail flick test to radiant heat, hot plate and writhling test induced by acetic acid were performed 60 min after the administration of the compounds or vehicle (DMSO for control group).

Mouse Tail Clip Test: This analgesic test was based on a method as described by Bianchi and Franceschini [23] and Dajani et.al.[24]A pressure standardized artery clip was placed approximately 2 cm from the base of tail and only the mice that responded to the clip placement by turning or biting at the clip within 15 s were used in this test.

Tail Flick Test to Radiant Heat: This test described by D'Amour and Smith [25] was done with a beam of high -intensity light focused on the dorsal surface of the tail. The response latency between the onset of the radiant heat stimulus and the movement of the tail out of the light beam of the apparatus (MAY, Commat, Ankara/Turkey) was determined.

The light intensity was set to provide a predrug response time of 2-4 s. A cut off 15 s was used in order to prevent damage to the tail.

Hot Plate Test: The test was based on that described by Eddy and Leimbach [26] and Noble et al[27]. A glass cylinder (height :16 mm , diameter: 16 mm) was used to keep the mouse on the heated surface of the plate which was kept at a temperature of 55+ 0.5 °C by using a thermoregulated water circulating pump. The latency period until the mouse licked a foot or jumped was registered by a means of a stopwatch (Cutoff time 45 s). The results were expressed as the percent of the maximal possible effect (%MPE).

%MPE= ((postdrug latency - predrug latency) / (cutoff time - predrug latency)) x 100

Forced Swimming Test: The procedure used was described by Porsolt et.al [28]. The apparatus consisted of a plexiglass cylinder (height: 25 cm, diameter:10 cm) containing 6 cm of water at 21-23 ^{0}C. 1 h after i.p. injection mice were dropped into the cylinder and left there for 6 min. Because little immobility was observed during the first 2 min only that occuring during the last 4 min. was evaluated. After a delay animals could stay immobile floating in the water, in an upright position making only very small movements necessary to keep their head above water. Total duration of immobility during the last 4 min was measured.

All the tests were conducted between 9 and 12 a.m. and all the results were expressed as means ± S.D. Statistical comparisons were performed by using Student's t test ($p < 0.05$).

Results and Discussion

The (RS)-Boc-Trp-Phe-NH_2(M0) and (R,S),(S,R),(R,R), and (S,S)-Boc-Trp-Phe-CO-morpholidide (M1-M4) were prepared by conventional solution -phase peptide synthesis [29] and the structures were elucidated by spectral analysis. All the compounds gave satisfactory spectral data.

The compounds thus obtained were subjected to antinociceptive tests including mouse tail clip, tail flick and hot plate and antidepressant effects were evaluated by Porsolt's Forced Swimming Test. The results were represented in Fig.1 and Fig. 2 respectively.

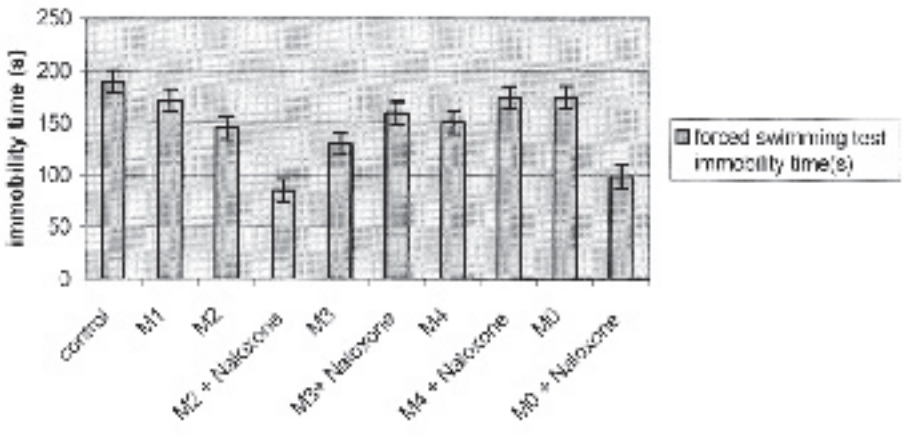

Figure 1

Anti-depressant effects of the compounds tested by Porsolt's Forced Swimming test
*$p < 0.05$ comparison with control grup + $p < 0.05$ comparison of the group administered by M1,M2,M3,M4 or M0 only with Naloxone administered groups.

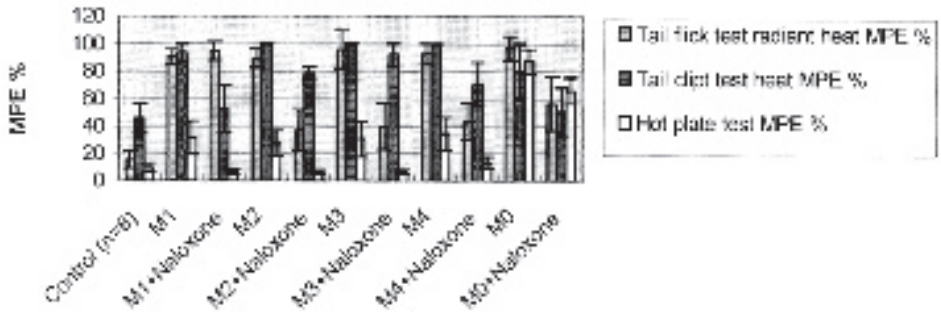

Figure 2

Anti-nociceptive effects of the compounds tested by tail clip and hot plate test *p < 0.05 comparison with control group + p < 0.05 comparison of the group administered by M1,M2,M3,M4 or M0 only with Naloxone administered groups.

According to the results above, it seems that the morpholidide derivatives (M1-M4) display non-stereoselective but significant naloxone-antagonised antinociceptive effect in the tail flick and tail clip tests compared to M0. On the other hand they have reduced effect in the hot plate test. While M0 was found inactive in the antidepressant activity test, the others proved to have stereoselective antidepressant effect, such that while M2, M3 and M4 reduced duration of immobility significantly, M1 did not. Thus it may be concluded that the chemical modification of the C-terminal Phe-NH_2 moiety of the dipeptide M0 by morpholine group does not alter antinociceptive activity in tail-flick and tail-clip tests but creates some reducement of activity in hot plate test. Considered together with the results of the antidepressant activity tests, it seems that such a modification brings about preference to δ-opioid receptor- mediated responses in the interaction route of CCK receptors with enkephelinergic system.

Abstract

In this study, the dipeptide M0, (R,S)-Boc-Trp-Phe-NH_2, designated as the chemical lead for the development of new CCK analogues, and the stereoisomers of its analog modified at the N-terminal by morpholine moiety which brings about sterical effect and adds an additional hydrogen binding site to the molecule. (R,R),(S,S),(R,S) and (S,R)-Boc-Trp-Phe-CO-morpholidide (M1-M4) were synthesized by conventional solution –phase peptide synthesis and structures elucidated by spectral analysis. The compounds thus obtained were subjected to antinociceptive tests including mouse tail clip, tail flick and hot plate and antidepressant effects were evaluated by Porsolt's Forced Swimming Test. The results elucidated by SAR (Structure activity relationship studies).

Keywords: Cholecystokinin (CCK), antinociceptive, antidepressant, dipeptoid.

Özet

Yeni Bir Kolesistokinin Dipeptoid Analoğunun ve Stereoizomerlerinin Sentezi, Antidepresan ve Antinosiseptif Etkilerinin İncelenmesi

Bu çalışmada, yeni CCK-analoglarının geliştirilmesinde öncü bileşik olarak belirlenmiş olan (R,S)-Boc-Trp-Phe-NH_2 (M0) bileşiğinin yapısı moleküle sterik etki ve ek hidrojen bağlanma bölgesi kazandırmak amacıyla modifiye edilerek, (R,R), (S,S), (R,S) ve (S,R)-Boc-Trp-Phe-CO-morfolidid (M1-M4) türevleri konvensiyonel sıvı faz peptit sentez metodu kullanılarak sentezlenmiş ve yapıları spektral analizlerle açıklanmıştır. Elde edilen bileşiklerin antinosiseptif etkileri tail clip, tail flick ve hot plate testleri ile, antidepresan etkileri ise Porsolt'un zorlu yüzdürme testi ile farelerde değerlendirilmiştir. Sonuçlar yapı-aktivite ilişkileri yönünden incelenmiştir.

Anahtar kelimeler : Kolesistokinin (CCK), antinosiseptif, antidepresan, dipeptoid.

Acknowledgements: This work was supported by Hacettepe University Research Fund (Project no: 9901301003).

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