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Effects of Pentadecapeptide BPC157 on Regional Serotonin Synthesis in the Rat Brain: α-Methyl-L-Tryptophan Autoradiographic Measurements

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Abstract

A novel pentadecapeptide, BPC157, was recently reported to have a large spectrum of in vivo activities, from anti-ulcer to central action on the brain dopaminergic system. The mechanisms of these actions are not well understood. In this study, the evaluation of the effects of acute and repeated administration of BPC157 on serotonin (5-HT) synthesis in the rat brain is reported. The α-[^{14}C]methyl-L-tryptophan (α-MTrp) autoradiographic method was used to measure regional 5-HT synthesis rates. In the first series of experiments, a single dose treatment of BPC157 (10 μg/kg) administered intraperitoneally 40 min before the α-MTrp tracer injection significantly reduced the regional rate of 5-HT synthesis in the dorsal thalamus, hippocampus, lateral geniculate body and hypothalamus. 5-HT synthesis rates in the substantia nigra reticulate and medial anterior olfactory nucleus in BPC157 treated rats were significantly higher than in the control rats. No significant change in the synthesis rate was observed in the raphe nuclei. In the second series of experiments, following a 7-day treatment with BPC157 (10 μg/kg; s.c.), a significant reduction in the 5-HT synthesis rate was observed in the dorsal raphe nucleus, and significant increases were observed in the substantia nigra, lateral caudate, accumbens nucleus and superior olive. This data suggests that BPC157, a

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gut peptide, influences brain 5-HT synthesis in rats, but we cannot determine, from this data, the mechanism of this action.

Keywords: α-methyl-L-tryptophan; Serotonin synthesis rate; Pentadecapeptide; BPC157; autoradiography

Introduction

Serotonin (5-HT) and both the central and peripheral systems have been implicated in various gastrointestinal disturbances (for review see, i.e., Stephens et al., 1990; Thompson, 1977; Gershon, 2003; Wood, 2001). However, the full significance of 5-HT involvement in these disturbances remains to be determined. Some antidepressants have been reported to protect gastrointestinal lesions in both experimental and clinical studies (Hernandez and Xue, 1989; Murison and Overmier, 1990; Ries et al., 1984; Somerville and Langman, 1983). However, the effect of antiulcer agents, administered peripherally, on 5-HT synthesis rates in the brain has not been fully investigated. A novel gastric pentadecapeptide, BPC157, is currently in clinical trials for inflammatory bowel disorder. BPC157 has the following amino acid sequence and molecular weight (MW): GlyGluProProProGlyLysProAlaAspAspAlaGlyLeuVal, MW = 1419. BPC157 has not been investigated for its possible influence on brain 5-HT synthesis. This peptide has been studied for its protective effects in the gastrointestinal tract (Sikirić et al., 1993; Paré and Kluczynski, 1994) and also for its beneficial effects in other organs (Sikirić et al., 1993, 1997b; Konjevoda et al., 1998; Seiwerth et al., 1997; Paré and Kluczynski, 1994; Bodis et al., 1997; Grabarevic et al., 1997). Recently, it was reported that an anti-ulcer pentadecapeptide, BPC157, had antidepressant effects during the Porsolt test and on chronic unpredictable stress in rats (Sikirić et al., 2000b). It was shown that BPC157 blocks the behaviour that is acutely produced by amphetamine in rats, as well as the development of haloperidol-induced supersensitivity to amphetamine in mice, suggesting effects on biogenic amine neurotransmission (Jelovac et al., 1998). It has also been reported that there is a significant pool of BPC157 from immunohistochemical staining in the stomach and brain (Sikirić et al., 1993). At this point it is not clear if this peptide is synthesized in the brain or if it enters the brain from the blood. Although the protective effect of BPC157 on gastric mucosa during stress has been related to the brain dopaminergic system (Sikirić et al., 1997a,c), the effects of this compound on brain 5-HT synthesis have not yet been determined. A relationship between ulcer disease and depression has been reported (Paré and Kluczynski, 1994; Stephens et al., 1990; Thompson, 1977), and this could relate to the action of this peptide on the brain monoaminergic systems. In this respect, externally administered BPC157 has been reported to reduce the duration of immobility to a greater extent than imipramine (Sikirić et al., 2000b).

The stable gastric pentadecapeptide BPC157 has been reported to have external wound healing capabilities, when given parentally, orally or locally (Sikirić et al., 2000a; Seiwerth et al., 1997; Konjevoda et al., 1998). It also modulates NO-synthesis (Sikirić et al., 1997b). In addition to the above mentioned effects this pentadecapeptide markedly inhibits influences of a stimulated release of dopamine (i.e., amphetamine-stereotyped behaviour (Jelovac et al., 1998), amphetamine-chronic disturbances (Sikirić et al., 2002), dopamine-receptor blockade such as neuroleptics-catalepsy (Jelovac et al., 1999a)), as well as dopamine-receptor supersensitivity (i.e., climbing behaviour that appears in

haloperidol treated mice subsequently challenged with amphetamine) (Jelovac et al., 1998). Likewise, it also antagonizes anxiogenic behaviour induced by amphetamine or other stimuli and attenuates withdrawal symptoms in diazepam chronically treated animals (Jelovac et al., 1999b). BPC157 antagonizes Porsolt's immobility/chronic stress freezing behaviour (Sikirić et al., 2000b), suggesting a possibility that its action is via the brain serotonergic system (Julien, 1998).

An autoradiographic method, using labelled α-[^{14}C]MTrp, has been developed to measure regional 5-HT synthesis rates in rat brains (Diksic et al., 1990; Nagahiro et al., 1990). This method is based on the unidirectional uptake (trapping) of α-MTrp, which is transported into the brain and converted, in part, to α-methyl-5-HT (Diksic et al., 1990; Nagahiro et al., 1990; Gharib et al., 1999). It has been shown that the metabolic pathway of α-MTrp follows (Diksic et al., 2000) the biosynthetic route of 5-HT from Trp (Steranka and Sanders-Bush, 1979).

In this study, the effects of acute and repeated treatment with pentadecapeptide BPC157 on 5-HT synthesis in rat brains using the α-[^{14}C]MTrp autoradiographic method was investigated.

Materials and methods

Animals

Forty eight (28 in each acute and chronic treatment groups) male Sprague-Dawley rats (Charles River), weighing 184–240 g, were housed in the animal facility (room temperature of 22°C with a 12 h day-night cycle) at least 3 days before the administration of the drug. The animals were housed as two per cage and were handled as little as possible. The animals were fasted overnight with water given ad libitum before the α-[^{14}C]MTrp autoradiographic experiments (overnight fasting is used to obtain a constant concentration of the plasma amino acids) and randomly selected in pairs of two (60 and 150 min experiments) for acute and chronic treatment/control groups. Acute and chronic experiments were not carried simultaneously. To avoid any influence of circadian rhythm on the results, the tracer was injected between 11:00 a.m. and 1:00p.m., and all rats were sacrificed between 1:00 p.m. and 3:00 p.m. The control animals were used in each treatment group for the purpose of preventing the seasonal influence or the effects of multiple handling in chronic experiments on the comparison of results. This means that control group was always handled in the same way as treatment group (e.g. injection, immobilization) and was done approximately in the same season of the year. Because of a difference in the handling in the chronic and acute experiments no direct comparison of the regional or global serotonin synthesis between chronic and acute experiments should be attempted. The body weight of each rat was recorded before the initial treatment of the drug and on the day of the surgical procedure. All animal use procedures were approved by the Institutional Animal Care Committee of McGill University and were performed according to the guidelines of the Canadian Council of Animal Care.

Administration of BPC157

The pentadecapeptide BPC157 (manufactured by Diagen, d.o.o., Ljubljana, Slovenia) was dissolved in saline (0.9% NaCl). The control rats received an equivolume of saline. In the acute treatments, BPC157 (14 rats), at a dose of 10 μg/kg, or saline (14 rats), at a volume of 2 ml/kg, was administered intraperitonelly (i.p.) 40 min before the injection of α-[^{14}C]MTrp. In the repeated treatment group, the

rats were injected subcontinuously (s.c.) with a 10 μg/kg/day dose of BPC157 (14 rats) or saline (14 rats) at a volume of 2 ml/kg for 7 days. The dose of the BPC157 was selected on the basis of the previous work in which it was shown that this dose produces anti-ulcer activity and has a central effect in rats (Sikirić et al., 1997a,b,2000b; Jelovac et al., 1998, 1999a; Bilic et al., 2001; Sikirić et al., 1999a,b). The volume of injection was standardised to be the same as that regularly used in the laboratory for tracer experiments (e.g. Nagahiro et al., 1990). The 5-HT synthesis rates in the repeated treatment group were determined 24 h following the last injection of BPC157 or saline. The main reason for using the rats in the tracer experiments 24 h following the last injection was the compatibility with our experimental procedure with other drugs (Diksic, 2001), as well as the fact that the measurements were performed at approximately the same time as when the repeated injections would have occurred.

Measurement of 5-HT synthesis

The 5-HT synthesis rate was determined using the autoradiographic α-[^{14}C]MTrp tracer method. Details of the autoradiographic α-[^{14}C]MTrp tracer method have been described in our previous publications (Nagahiro et al., 1990; Diksic, 2001). The rats were on halothane anaesthesia (approximately 1%) and plastic catheters were inserted into the right femoral artery (for blood sampling) and vein (for tracer injection). The rats were allowed to recover for one hour from the anaesthesia which was usually administered for 20 to 30 min, and the lower half of the body was immobilized with a loose-fitting plaster cast on a lead brick. Body temperature was measured with a flexible thermo-probe inserted over 6 cm into the rectum and maintained at 37 ± 0.5°C with a heating lamp throughout the experimental procedure. Throughout all of the experiments, the physiological variables (arterial pO2, pCO2, pH and hematocrit) were measured. These measurements were done to assess if the general state of rats on different experimental days were about the same. Any rat with greatly different blood gases from normal would be removed from the group, however this occurs very rarely and did not occur in experiments reported here. Approximately two hours after the surgical procedure, 30 μCi of α-[^{14}C]MTrp (specific activity of 55 mCi/mmol; synthesized by us using the procedure previously described (Mzengeza et al., 1993)) in 1 ml of saline was administered at a constant rate through the femoral vein over a period of 2 min. Arterial blood samples were collected at progressively increased time intervals up until the time of decapitation. Twenty μL of plasma were taken and the radioactivity was measured using liquid scintillation counting to obtain the input function. The animals were decapitated 60 or 150 min following the tracer injection to permit utilization of the model as described in Nagahiro et al. (1990), and their brains were rapidly removed and frozen in isopentane (−25°C). The brains were coronally sectioned (30 μm thick) in a cryostat (−25°C) and thaw-mounted on glass slides. The brain sections were dried on a hot plate at 60°C and exposed to x-ray film along with [^{14}C]-methyl methacrylate standards (American Radiolabel Co., St. Louis, MO, USA) in x-ray cassettes for 3 weeks to obtain the autoradiograms. The resultant images on the x-ray film were analysed using a microcomputer-based image analysing system (MCID/M4-Image Analysis System, Imaging Research Inc., Canada) and tissue equivalent calibrated standards.

Six plasma samples were taken at different times to determine the total (plasma deproteinized with 20% tri-chloroacetic acid) and free (non-albumin-bound; ultra-filtrate) Trp concentrations in the plasma. Total and free Trp concentrations were measured by a high performance liquid chromatography method using fluorescence detection (Takada et al., 1993). Distribution volumes (VD*;ml/g) were calculated by dividing the tissue tracer concentration [C*(t); nCi/g] with the plasma tracer concentration at the end of

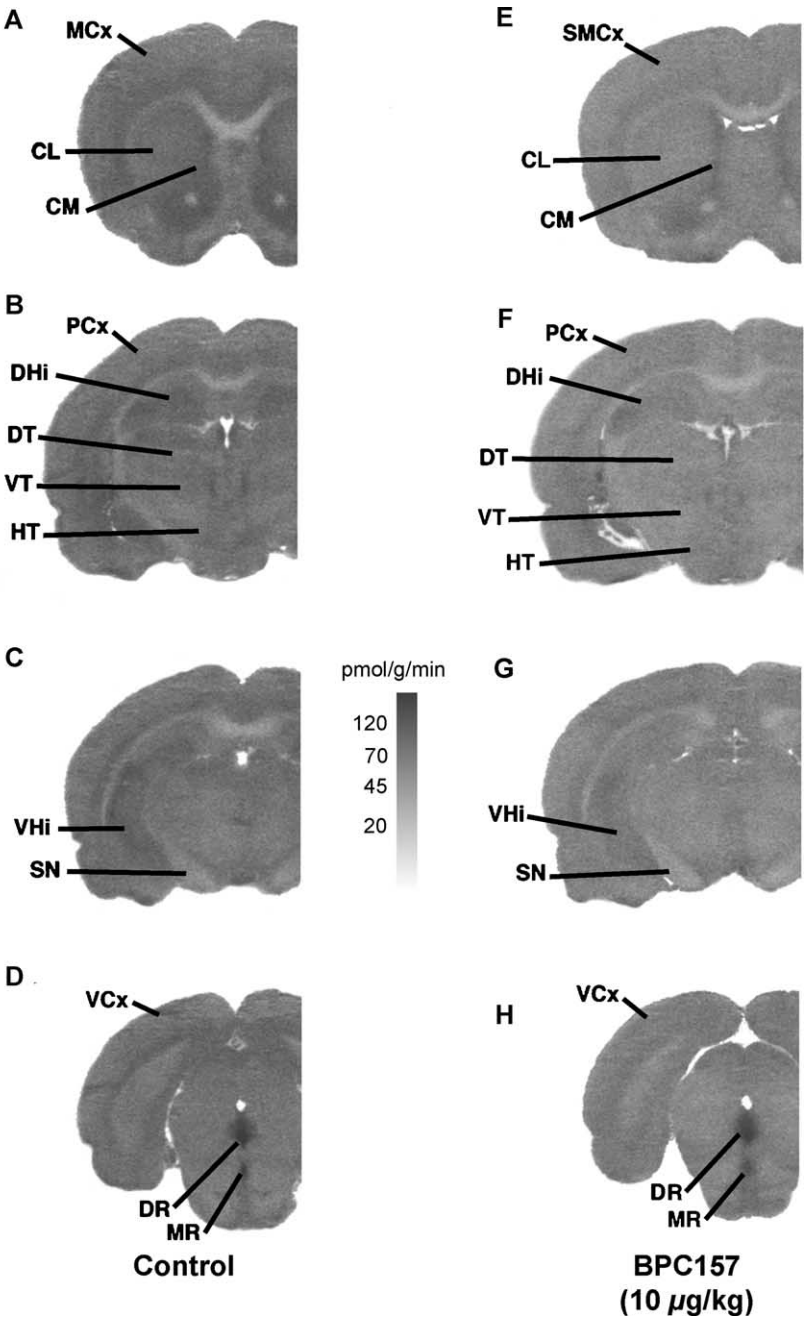

Fig. 1. Representative autoradiograms obtained in the rats treated with BPC157 (10 μg/kg, i.p.) and the rats in the control group treated with saline in an acute experimental protocol are shown. The autoradiograms shown here are taken from the rat brain 150 min following the tracer injection. The brain slices were exposed for 3 weeks and digitized. The meaning of the structure abbreviations are: DR, raphe dorsal; MR, median; VCx, cortex visual; PCx, cortex parietal; SMCx, cortex sensory-motor; CL, caudate lateral; CM, caudate medial; TV, thalamus ventral; DL, thalamus dorsal; Dhi, hippocampus dorsal; Vhi, hippocampus ventral; PB, pineal body; SN, substantia nigra; and AC, nucleus accumbens.

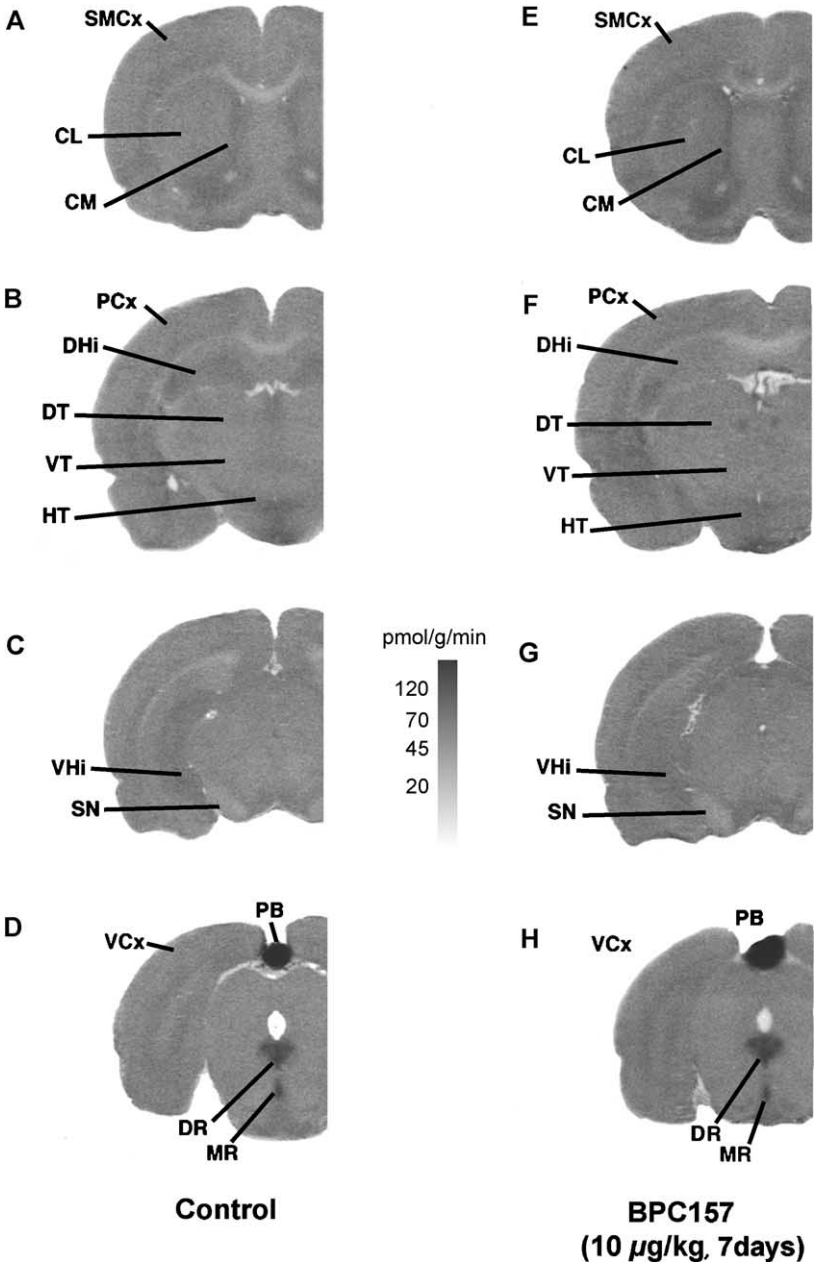

Fig. 2. Representative autoradiograms obtained in the rats treated with BPC157 (10 μg/kg/day, s.c. for seven days) and the rats in the control group treated with saline in a chronic experimental protocol are shown. The autoradiograms shown here are from the rat brain 150 min following the tracer injection. The brain slices were exposed for 3 weeks and digitized. The meaning of the structure abbreviations are: DR, raphe dorsal; MR, median; VCx, cortex visual; PCx, cortex parietal; SMCx, cortex sensory-motor; CL, caudate lateral; CM, caudate medial; TV, thalamus ventral; DL, thalamus dorsal; Dhi, hippocampus dorsal; Vhi, hippocampus ventral; PB, pineal body; SN, substantia nigra; and AC, nucleus accumbens.

the experiment [Cp*(T); nCi/ml]. As shown earlier (Diksic et al., 1990; 1995), it is reasonable to assume that VD*-s are linearly related to the exposure time Θ [$\Theta = {}_0\int^T Cp^*(t)/Cp^*(T)dt$; min], where Cp*(t) (nCi/ml) is the tracer concentration in the plasma as a function of time. From this linear relationship, the slope is equal to the trapping constant of α-MTrp (K*;ml/g/min). The rate of 5-HT synthesis R; pmol/g/min) was calculated as R = Cp K* / LC, where Cp is the plasma concentration of free Trp (pmol/ml), and the LC is a factor which converts the brain's trapping of α-MTrp (K*) into the regional uptake constant for the metabolism of Trp to 5-HT (Diksic, 2001). The LC (0.42±0.07) measured in vivo in the rat brain was used (Vanier et al., 1995).

The 5-HT synthesis rates for the presentation in Figs. 1 and 2 were calculated by assuming an average value of the precursor pool, as previously discussed (Diksic et al., 1995) using images obtained in rats killed 150 min after tracer injection. The main reason to use images from rats killed after 150 min instead those killed 60 min following tracer injection is a better visual delineation of the brain structures in the former one. However, in general a good agreement is expected between syntheses calculated from images obtained from rats killed 60 and 150 min after tracer injection.

Table 1
5-HT synthesis rate in the rat brains 40 min after the treatment of BPC157 (10 μg/kg, i.p.)

Structures	Control (n = 14)	BPC157 (n = 14)	% Change (*t* value)
Dorsal raphe nucleus	236 ± 25	235 ± 26	0
Medial raphe nucleus	151 ± 19	150 ± 15	−1
Magnum raphe nucleus	84 ± 11	83 ± 9	−2
Visual cortex	56 ± 6	53 ± 7	−5
Auditory cortex	56 ± 8	55 ± 7	−2
Parietal cortex	53 ± 8	51 ± 8	−3
Sensory-motor cortex	50 ± 7	46 ± 10	−11
Frontal cortex	49 ± 6	51 ± 9	+3
Substantia nigra reticulata	36 ± 7	47 ± 7	+32**(+3.74)
Substantia nigra compacta	51 ± 8	52 ± 7	+2
Globus pallidus	60 ± 6	56 ± 8	−7
Caudate (lateral)	57 ± 7	57 ± 9	−1
Caudate (medial)	69 ± 9	65 ± 9	−6
Thalamus (ventral)	55 ± 8	48 ± 8	−13
Thalamus (dorsal)	53 ± 7	44 ± 8	−15*(−2.40)
Hippocampus (ventral)	79 ± 9	70 ± 8	−11*(−2.33)
Hippocampus (dorsal)	79 ± 10	63 ± 8	−20*(−4.14)
Amygdala	71 ± 5	67 ± 6	−6
Medial geniculate body	54 ± 8	54 ± 7	−1
Lateral geniculate body	59 ± 8	49 ± 8	−16**(−2.91)
Ventral tegmental area	66 ± 9	62 ± 7	−5
Medial forebrain bundle	59 ± 8	56 ± 11	−5
Hypothalamus	61 ± 7	54 ± 9	−12**(−2.16)
Superior colliculus	49 ± 8	49 ± 8	+1
Accumbent nucleus	86 ± 8	80 ± 9	−7
Medial anterior olfactory nucleus	61 ± 7	71 ± 6	+16**(3.34)
Superior olive	42 ± 9	46 ± 11	+8
Pineal body	863 ± 103	834 ± 104	−3

Values are presented as ± calculated from the variance of the linear regression.
Differences with $p < 0.05$ were considered to be significant with 26 degrees of freedom (*$p < 0.05$, **$p < 0.01$).

Statistical analysis

Two-tailed t-tests were used to evaluate the differences between the treatment and respective control groups. The main effect was evaluated by comparing the ratios of 5-HT synthesis in the control and treatment groups. The ratios between the control and treated rats were compared using the one sample two-tailed t-test with a null hypothesis of the ratio equalling one, with a standard deviation of zero. If there was no difference of treatment, the average ratio should not be different from one. After the main effect was found to be significant ($p < 0.05$), the individual structures in the treatment groups were compared to those in the control groups using the two-tailed t-test (note that only two groups were involved in the comparisons.) The t-values and identification for those which were significant at $p < 0.05$ are provided in the Tables 1 and 2. There is therefore a possibility that two structures identified as being significantly different may be so because of chance. Despite this, one can conclude that a large number of structures are properly identified as significantly different. All statistical

Table 2
5-HT synthesis rate in the rat brains after the 7-day treatment of BPC157 (10 μg/kg/day, s.c.)

Structures	Control (n = 14)	BPC157 (n = 14)	%Change (*t*-value)
Dorsal raphe nucleus	208 ± 19	192 ± 26	−7*(−2.11)
Medial raphe nucleus	130 ± 15	129 ± 17	−1
Magnum raphe nucleus	60 ± 6	59 ± 12	−2
Visual cortex	37 ± 6	39 ± 9	+7
Auditory cortex	39 ± 6	41 ± 9	+6
Parietal cortex	31 ± 6	34 ± 9	+10
Sensory-motor cortex	36 ± 7	33 ± 9	−8
Frontal cortex	34 ± 8	34 ± 8	0
Substantia nigra reticulata	26 ± 7	37 ± 10	+42**(+3.43)
Substantia nigra compacta	34 ± 7	42 ± 8	+23*(+2.73)
Globus pallidus	43 ± 6	48 ± 9	+12
Caudate (lateral)	42 ± 7	49 ± 9	+16*(+2.30)
Caudate (medial)	55 ± 7	53 ± 8	−4
Thalamus (ventral)	34 ± 6	37 ± 9	+7
Thalamus (dorsal)	33 ± 6	33 ± 9	+2
Hippocampus (ventral)	57 ± 7	59 ± 9	+3
Hippocampus (dorsal)	56 ± 7	54 ± 9	−2
Amygdala	50 ± 6	50 ± 9	+2
Medial geniculate body	37 ± 7	43 ± 8	+15
Lateral geniculate body	38 ± 6	39 ± 9	+4
Ventral tegmental area	46 ± 7	52 ± 9	+13
Medial forebrain bundle	41 ± 7	44 ± 8	+7
Hypothalamus	40 ± 6	42 ± 9	+5
Superior colliculus	34 ± 7	35 ± 8	+4
Accumbent nucleus	62 ± 7	68 ± 7	+9*(+2.11)
Medial anterior olfactory nucleus	60 ± 6	56 ± 7	−7
Superior olive	28 ± 8	34 ± 7	+23*(+2.11)
Pineal body	781 ± 98	712 ± 113	−9

Values are presented as ± calculated from the variance of the linear regression.
Differences with $p < 0.05$ were considered to be significant with 26 degrees of freedom (*$p < 0.05$, **$p < 0.01$).

calculations were done within Excel spread sheet. The number of the degrees of freedom in two-tailed t-test was 26.

Results

There was no significant difference in the physiological parameters between the treated and control rats. The plasma concentration of free tryptophan in the single dose experiments in the control group was 13.1 ± 3.3 (nmol/mL) and in the treatment group, it was 12.6 ± 3.4 (nmol/mL). The plasma concentration of total tryptophan was 92 ± 11 (nmol/mL) and 86 ± 18 (nmol/mL) in the control and treated rats in the acute experiment, while in the chronic experiment, the concentration was 64 ± 15 (nmol/mL) and 72 ± 19 (nmol/mL) in the control and treated rats, respectively. In the repeated injection experiments, the free tryptophan concentration in the plasma was 12.1 ± 3.0 (nmol/mL) and 11.6 ± 3.9 (nmol/mL) in the control and treated rats, respectively. There were no significant differences in the plasma Trp concentration between any of these groups, however only the treatment and respective controls were compared.

The representative autoradiograms, illustrating the regional 5-HT synthesis rates in the rat brain obtained 150 min following the injections of α-[^{14}C]MTrp, are shown in Figs. 1 (acute experiment) and 2 (chronic experiment). Increased 5-HT synthesis can be noticed in the brain structures known to contain a large concentration of serotonergic cell bodies (e.g. raphe nuclei) or structures receiving a large density of serotonergic projections (e.g. cortical layer VI). The 5-HT synthesis rates in 28 structures of the rat brain following acute administration of BPC157 or saline are provided in Table 1. The overall synthesis rate in the BPC157 treated rats was significantly lower than in the control group ($p = 0.0399$; one sample t-test on the ratio: ratio = 1.04 ± 0.10; N = 27). Significant decreases in the 5-HT synthesis rates were observed in the dorsal thalamus (−15%), dorsal (−20%) and ventral (−11%) hippocampus, lateral geniculate body (−16%) and hypothalamus (−12%). The 5-HT synthesis rates in the substantia nigra reticulata (+32%) and medial olfactory nucleus (+16%) in the BPC157 treated rats was significantly higher than in the control rats.

The regional 5-HT synthesis rates in the rat brain 24 h following treatment of BPC157 for 7 days are provided in Table 2. The synthesis rate in the repeated BPC157 group is, overall, significantly greater than in the control rats ($p = 0.0064$; one sample t-test on the ratio: ratio = 0.95 ± 0.09; N = 27). This repeated treatment with BPC157 (10 μg/kg/day for 7-days) significantly increased the rate of 5-HT in the following regions: the substantia nigra reticulata (+42%) and compact (+23%), lateral caudate (+16%), accumbens nucleus (+9%) and superior olive (+23%). It should be noted that there was a significant reduction of 5-HT synthesis in the dorsal raphe nucleus, while the majority of structures showed an increased synthesis after treatment with BPC157.

The synthesis is the highest in the pineal body in both acute and chronic treatments, as usually has been observed in previous experiments (e.g. Diksic et al., 1995; Tohyama et al., 2002).

Discussion

The data presented here suggest that gastric pentadecapeptide BPC157, when administered peripherally, has region specific influences on brain 5-HT synthesis. In general, the effect of BPC157

on brain 5-HT synthesis appears to be different following acute and chronic treatments. Following acute treatment, a slight, but not significant, decrease was observed in a large number of brain structures. 5-HT synthesis decreases overall following acute administration (Table 1), but increases following chronic treatment (Table 2), by comparing treatment and respective control groups. An increase in 5-HT synthesis in the brain of immobilized rats has been reported previously (Curzon and Green, 1969). However, in the present experiments, it is unlikely that immobilization would produce confounding effects because the control group that was injected with saline was also immobilized for the same period of time as the treated groups. As mentioned in the methods direct comparisons between syntheses in the acutely and chronically treated rats were not done.

Following acute administration, the synthesis is significantly increased in the medial anterior olfactory nucleus and substantia nigra reticulata, while there is a significant decrease in the globus pallidus, dorsal and ventral hippocampus, dorsal thalamus, lateral geniculate body, and hypothalamus. The substantia nigra's (compacta and reticulata) structure is potentially important because 5-HT synthesis was significantly increased following both acute and chronic pentadecapeptide BPC157 treatments. It should be noted that increased synthesis in the substantia nigra reticulata is present, even when there is an overall decrease in brain 5-HT synthesis following acute treatment (Table 1). In the same brain structure an increase of the synthesis was observed in chronically treated rats (Table 2). In addition to a significant increase in the substantia nigra increases were observed in the lateral caudate and accumbens nucleus, while there was a significant decrease observed in the dorsal raphe. The importance of the substantia nigra may be related to the fact that this BPC157 antagonized disturbances induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a neurotoxin known to be specifically damaging to dopamine substantia nigra cell bodies (Sikirić et al., 1999b). This structure has a dense presence of dopaminergic neurons and receives dense projections of the serotonergic systems (Azmitia and Segal, 1978). It has been previously reported that BPC157 has a significant effect on the brain dopaminergic system (Jelovac et al., 1998, 1999a,b). Dense projections of both the monoaminergic systems and a strong interaction between those two systems suggest that the effects on brain serotonin synthesis observed in the present study could be related, in part, to the BPC157 effects on the brain dopaminergic system. Observed decrease in the dorsal raphe could be also a result of an interaction between dopaminergic and serotonergic systems. The effects of BPC157 reported in this study do not resemble the results obtained with any other serotonergic drug using this method (Diksic, 2001; Diksic and Young, 2001; Tohyama et al., 2002). This may suggest that even though BPC157 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 BPC157 action probably differs greatly. There is not yet any data to suggest that BPC157 has any specific serotonergic properties. However, external administration of BPC157 reduced the duration of immobility to a greater extent than imipramine, which suggests the possible involvement of the brain serotonergic and noradrenergic systems (Sikirić et al., 2000b). This peptide is found in the brain but there is no data to prove that following peripheral administration, its action occurs in the brain on the brain serotonergic system. It is also possible that this peptide acts on the brain serotonergic system indirectly from a binding site in the gut at some visceral receptive relay (Aziz and Thompson, 1998) of the central nervous system (e.g. Jelovac et al., 1998, 1999a,b).

Indeed, the effects described here are obtained in normal rats and under normal conditions. The observed data, in conjunction with the reported effects of BPC157 on the brain DA system, suggest that there is probably a complex DA-5-HT interaction occurring under the influence of this peptide.

There is the possibility that gut-brain interaction is responsible for this centrally observed effect. It is also possible that this pentadecapeptide has similar effects on the brain serotonergic system under certain altered conditions, but this hypothesis needs further investigation. On the other hand, gastric pentadecapeptide BPC157, which counteracts behavioural disturbances induced by various agents (Sikirić et al., 2002; Jelovac et al., 1999b), does not alter behaviour in normal animals (Sikirić et al., 2002).

In summary, BPC157 administered intraperitoneally has a region specific effect on brain 5-HT synthesis. There is no data at this time to prove the suggestion based on the central action observed in the present study that this peptide enters the brain. Perhaps it enters through circumventricular organs (as angiotensin II) and/or initiates its effect at the periphery at some visceral receptive relay of the central nervous system. Regardless whether the action is direct or indirect some data suggest that its action is through some visceral site of the central nervous system (Koob and Bloom, 1983). However the full elucidation of the mechanism(s) requires further experiments.

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