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SCIENCE DIRECT
Bioorganic & Medicinal Chemistry Letters
ELSEVIER
Bioorganic & Medicinal Chemistry Letters 14 (2004) 369–372

Synthesis and biological characterisation of [^{3}H]BBL454, a new CCK_2 selective radiolabelled agonist displaying original pharmacological properties

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Received 9 April 2003; revised 17 July 2003; accepted 3 November 2003

Abstract—[^{3}H]BBL454, a new CCK_2 selective tritiated agonist was prepared via the reductive tritiation of a 5-aminopentyn-1-yl moiety introduced on the N-terminal end of a pentapeptide derivative of cholecystokinin. The binding properties of this labelled compound were determined on CHO cells transfected with the rat CCK_2 receptor. [^{3}H]BBL454 is able to discriminate two affinity states of the CCK_2 receptor a supplementary indication of its validity for further exploring the heterogeneity of this receptor.

1. Introduction

The peptide cholecystokinin (CCK-8, Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2), both a neurotransmitter and a hormone, is involved in several physiological processes including regulation of appetite and gastrointestinal functions, anxiety, cognitive processes and analgesia.[1,2] All these effects result from the interaction of cholecystokinin with two seven transmembrane receptors termed CCK_1 and CCK_2 (formerly CCK-A and CCK-B respectively), the latter being widely distributed throughout the brain.[2] Although no evidence for a structural heterogeneity of the CCK_2 receptors has been obtained to date,[3] numerous data suggest that CCK_2 agonists can be separated into two classes, regarding their pharmacological properties in vivo.[4] On the one hand, most CCK_2 agonists including the endogenous peptides CCK-8 and CCK-4 are associated with the so-called 'CCK_{2A}' profile (formerly 'CCK-B1′), characterised by anxiogenic properties and a negative or non-measurable effect on memory processes. On the other hand, the heptapeptide BC264 [Boc-Tyr(SO_3H)-gNle-mGly-Trp-NMeNle-Asp-Phe-NH_2, Table 1][5] was the only available CCK_{2B} agonist for a decade; this widely studied compound is non-anxiogenic,[6] increases the exploratory behaviour of rats,[7] stimulates dopamine release in vivo[8] and reinforces working memory.[9] Recently, a new series of BC264-derived pentapeptides[10] was developed by a rational process, in which the lead compound RB400 (HO_2C-CH_2-CO-Trp-NMeNle-Asp-Phe-NH_2, Table 1), and some of its derivatives were shown to share the CCK_{2B} pharmacology.[11] In particular, the most potent compound, BBL454 [H-$(CH_2)_5$-NHCO-CH_2CO-Trp-NMeNle-Asp-Phe-NH_2] shares the favourable properties of BC264 (increase of working memory, for instance), but at doses 10–100 times lower (as low as 0.03 μg/kg after ip injection in the rat), and remains active in a large range of doses[11] (0.03–300 μg/kg in the open-field test). In contrast, BC264, the former CCK_{2B} compound, is not satisfying from this point of view, as it is only active in a small concentration range[9] (i.e., in a two-trial memory test, BC264 is only active at 3 μg/kg, but not at any dose lower nor higher).

Unfortunately, numerous experiments in several receptor preparations (cortical membranes from diverse species or CHO cells stably transfected with the rat CCK_2 receptor) have been so far unsuccessful in the aim of establishing a biochemical discrepancy between CCK_{2A}

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doi:10.1016/j.bmcl.2003.11.001

Table 1. Essential biological data of reference CCK_2 agonists[20]

Compd	K_i (CCK_2)	K_i (CCK_1)	Pharmacology
CCK-8 **Asp-Tyr(SO_3H)-Met-Gly**-Trp-Met-Asp-PheNH_2	0.6 nM	0.7 nM	Mixed CCK_1/CCK_{2A} agonist
BC264 **Boc-Tyr(SO_3H)-gNle-mGly**-Trp-NMeNle-Asp-PheNH_2	0.31 nM	96 nM	CCK_{2B} agonist
RB400 **HO_2C-CH_2-CO**-Trp-NMeNle-Asp-Phe-NH_2	0.75 nM	> 3 μM	CCK_{2B} agonist
PJC-42 **$(CH_3)_3C$-OCO**-Trp-NMeNle-Asp-Phe-NH_2	2.6 nM	> 5 μM	CCK_{2A} agonist
BBL454 **H-$(CH_2)_5$NHCOCH_2CO**-Trp-NMeNle-Asp-PheNH_2	2.66 nM	3.64 μM	CCK_{2B} agonist

and CCK_{2B} agonists. However, binding studies using CCK_2 agonists or antagonists[12] support the hypothesis that the pool of CCK_2 receptors could be constituted of several affinity states. Additional data also show that multiple transduction systems are associated with CCK_2 receptors.[13]

Therefore, the design of more 'selective' CCK_{2A} or CCK_{2B} compounds appeared as mandatory to further study the relevance of CCK_{2A}/CCK_{2B} discrimination in vitro. CCK_{2B} selectivity is a difficult parameter to determine, but we hypothesised that the width of the concentration range of activity of CCK_{2B} agonists in vivo could be an indication of this parameter. BC264, which is generally only active at a single dose in vivo would in this case be classified as a poorly selective CCK_{2B} agonist, whereas BBL454, having a large range of activity, could be considered highly CCK_{2B}-selective. Thus, a radiolabelled analogue of BBL454 was of great interest, all the more since desirable pharmacological effects displayed by BBL454 remain associated with some unexplained issues. In particular, why is BBL454 100 times more active than BC264 in the open-field test using rats while its affinity is 10 times lower on rat CCK_2 receptors? Variations in the bioavailability of the compounds, which a radiolabelled analogue could help to determine, might be a convenient explanation for these observations. This prompted us into the design of a labelled derivative of BBL454.

2. Strategy and synthesis

The extensive structure–affinity relationships obtained with derivatives of RB400[10] and the in vivo study of several representative compounds has proved that slight structural differences could lead to a change of the pharmacological profile from CCK_{2A} to CCK_{2B}. Thus, it seemed crucial for our purposes that the introduction of a radioisotope should modify as little as possible the structure of BBL454. Previous works on tritiated CCK agonists had used the unsaturated analogue of norleucine L-2-amino-4-hexynoic acid[14] or the phenylalanine precursor L-3(3′,4′,5′-tribromophenyl)alanine[15] to introduce labels by means of catalytic reduction of the precursors by tritium gas. In our case, the presence of both *N*-methylnorleucine and phenylalanine in the sequence of BBL454 allowed us to apply this strategy, but this necessitated the preparation of unnatural, enantiomerically pure aminoacids, whose reported synthesis gave poor yields.[14,15] Thus, an unsaturated N-terminal analogue of BBL454 appeared as much more promising, since its reduction would lead to [^{3}H]BBL454 without any structural modification but the substitution of hydrogen atoms by their isotopes.

The key step in the chosen strategy was thus the preparation of any unsaturated five-carbon-length linear primary amine. Obviously, the more insaturated bonds that are present in the resulting precursor, the higher the specific activity that should be obtained by catalytic tritiation. Thus, 1-aminopentynes were chosen as potential intermediates.

Preliminary attempts to convert commercially available 3-pentyn-1-ol into the corresponding amine failed, probably due to the susceptibility of intermediate activated alcohols to elimination and/or nucleophilic substitution, even by triethylamine. Therefore, a chloropentyne was found to be preferable than a pentynol as starting material. Thus, 5-chloropent-1-yne (**3**) was converted in quantitative yields to *N*-(pent-4-yn-1-yl)-phthalimide (**4**) by nucleophilic substitution using sodium iodide and potassium phthalimide; the resulting compound was purified by recrystallization as previously described.[16] Surprisingly, hydrazinolysis under the conditions described by the same authors afforded a complex mixture of compounds, from which the desired 1-amino-pent-4-yne could neither be isolated as such, nor as the hydrochloride salt. Alternative attempts at deprotection of the phthalimide group in acidic (HCl/AcOH) medium led to deprotected compounds showing addition of HCl to the triple bond.

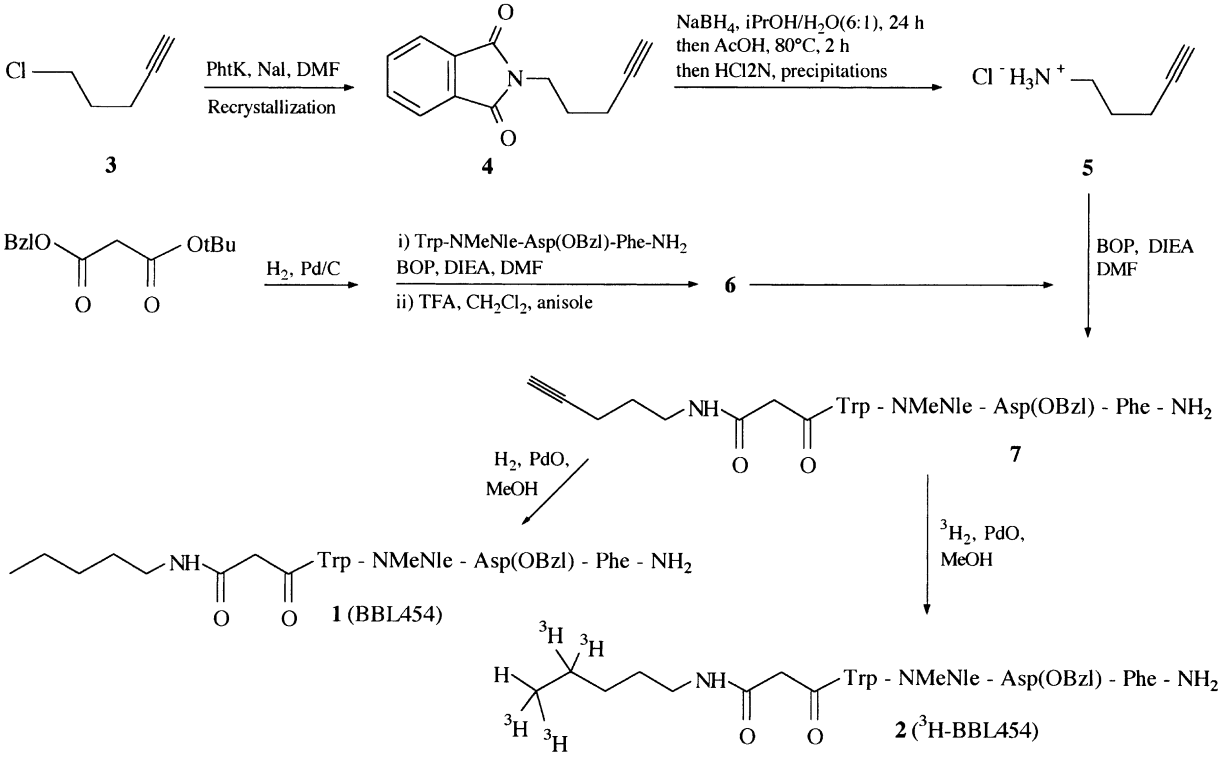
Therefore, milder deprotection conditions were used involving reduction of the phtalimide by sodium borohydride in a water/isopropyl alcohol mixture followed by treatment with diluted acetic acid.[17] Purification, as published,[17] of the compound by ion-exchange chromatography was avoided because of the suspected high

volatility of the expected amine, which would have made it difficult to isolate from the elution solvent. Thus, the final reaction mixture was acidified with hydrochloric acid, filtered and precipitated in ether to separate inorganic and organic boron salts. Smooth evaporation of the ethereal solution provided the amine **5** only contaminated with remaining boron salts which could not be completely eliminated (thus leading to an apparent overall yield exceeding 100%), but which did not compromise the following steps of synthesis. Next, **5** was coupled with the pentapeptide analogue **6** (obtained by condensation of mono-*tert*-butyl malonate with the tetrapeptide Trp-NMeNle-Asp(OBzl)Phe-NH_2 followed by deprotection of the *tert*-butyl ester with trifluoroacetic acid) yielding the unsaturated precursor of BBL454, **7**. Catalytic hydrogenation at atmospheric pressure of **7** in methanol, with palladium oxide as catalyst, gave BBL454 (**1**)[18] in 100% raw yield (without purification); tritiation of **7** under the same conditions led to [^{3}H]BBL454 (**2**) with an estimated specific activity of 50 Ci/mmol (Scheme 1).

3. Biochemical characterization

Cold BBL454 was assayed following standard protocols[10] by competition of [^{3}H]pCCK-8 from membranes of CHO cells transfected with CCK_2 receptors ($K_i = 2.7 \pm 0.3$ nM), and proved to behave as a full agonist of inositol phosphate production ($EC_{50} = 2 \pm 0.3$ nM) and arachidonic acid liberation ($EC_{50} = 6.9 \pm 0.8$ nM) by intact transfected CHO cells (data not shown). Then, the binding properties of the new radiolabelled agonist [^{3}H]BBL454 were assayed on intact CHO cells following a protocol described previously.[19] Cold saturation experiments conducted with BBL454 competing with [^{3}H]BBL454 proved that [^{3}H]BBL454 binds to two sites in CHO cells, in a specific and saturable way. Scatchard analysis of these results reveals a high affinity and low populated binding site ($K_{D1} = 0.71 \pm 0.17$ nM, $B_{max1} = 120 \pm 16$ fmol/mg of protein) and a low affinity and high populated binding site ($K_{D2} = 194 \pm 76$ nM, $B_{max2} = 4.41 \pm 0.71$ pmol/mg of protein). A representative Scatchard diagram of these experiments is presented in Figure 1. That BBL454 is able to discriminate two affinity states of the CCK_2 receptor is a supplementary indication as to its validity for further exploring the heterogeneity of these receptors. Therefore, competition experiments using this new radiolabelled agonist and various standard CCK_2 ligands are now under progress.

Owing to its remarkable pharmacological properties in vivo, the CCK_{2B} agonist BBL454 emerges as a valuable tool for studying the functional heterogeneity of CCK_2 receptors, and merely as a major starting point for the research of compounds exerting stimulant and memory reinforcing. Its tritiated analogue will now be used to assay the bioavailability of BBL454 in vivo, and to determine, via autoradiographic studies, the location of its sites of action. Its is also firmly hoped that this radioligand will be useful for designing a high throughput biochemical test for discriminating easily and rapidly CCK_{2A} from CCK_{2B} agonists; its capacity to discriminate between two affinity states of the receptor is a first indication that it may be useful for such analyses.



Scheme 1. Synthesis of [^{3}H]BBL454.

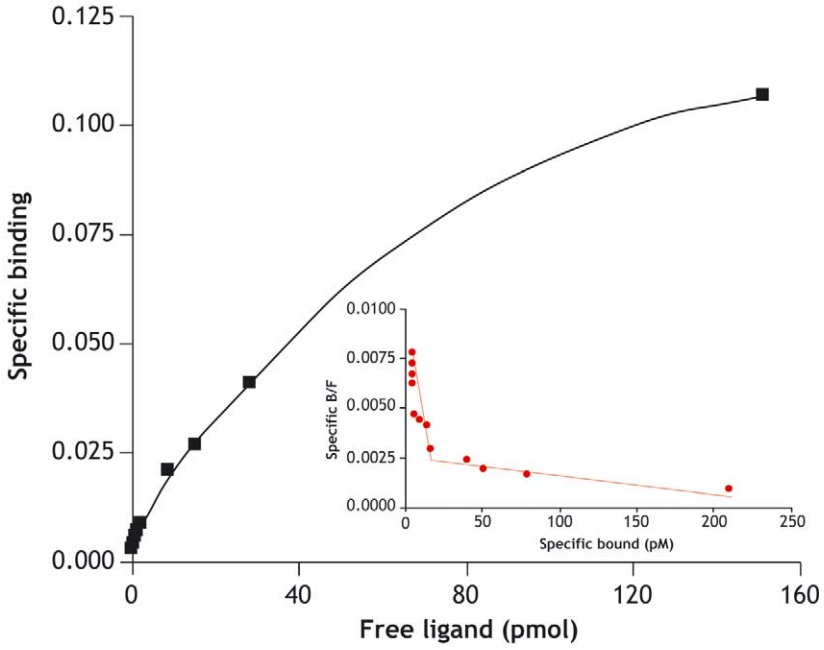


Figure 1. Saturation of BBL454 on CHO cells and corresponding Scatchard diagram.

Acknowledgements

The authors wish to acknowledge Michel Vidal for numerous helpful technical suggestions and support.

References and notes

1. Reviewed in: Crawley, J. N.; Corwin, R. L. *Peptides* **1994**, *15*, 731.
2. Reviewed in: Noble, F.; Wank, S. A.; Crawley, J. N.; Bradwejn, J.; Seroogy, K. B.; Hamon, M.; Roques, B. P. *Pharmacol. Rev.* **1999**, *51*, 745.
3. Reviewed in: Noble, F.; Roques, B. P. *Prog. Neurobiol.* **1999**, *58*, 349.
4. Reviewed in: Daugé, V.; Léna, I. *Neurosci. Biobehav. Rev.* **1998**, *22*, 815.
5. Charpentier, B.; Durieux, C.; Pélaprat, D.; Dor, A.; Reibaud, M.; Blanchard, J. C.; Roques, B. P. *Peptides* **1988**, *9*, 835.
6. Derrien, M.; McCort-Tranchepain, I.; Ducos, B.; Roques, B. P.; Durieux, C. *Pharmacol. Biochem. Behav.* **1994**, *49*, 133. Charrier, D.; Dangoumau, L.; Puech, A. J.; Hamon, M.; Thiébot, M. H. *Psychopharmacology (Berl)* **1995**, *121*, 127.
7. Ladurelle, N.; Roques, B. P.; Daugé, V. *J. Neurosci.* **1995**, *15*, 3118.
8. Ladurelle, N.; Keller, G.; Blommaert, A.; Roques, B. P.; Daugé, V. *Eur. J. Neurosci.* **1997**, *9*, 1804.
9. Léna, I.; Simon, H.; Roques, B. P.; Daugé, V. *Neuropharmacology* **1999**, *38*, 543.
10. Million, M. E.; Léna, I.; Danascimento, S.; Noble, F.; Daugé, V.; Garbay, C.; Roques, B. P. *Lett. Pept. Sci.* **1997**, *4*, 407. Bellier, B.; Million, M. E.; Danascimento, S.; Meudal, H.; Kellou, S.; Maigret, B.; Garbay, C. *J. Med. Chem.* **2000**, *43*, 3614.
11. Bellier, B. PhD Thesis, Université Paris V, 2000. Bellier, B.; Crété, D.; Million, M. E.; Beslot, F.; Bado, A.; Garbay, C.; Daugé, V. *Eur. J. Pharmacol.* submitted.
12. Reviewed in: Bellier, B.; Gigoux, V.; Garbay, C. *Curr. Med. Chem. CNSA* **2001**, *1*, 209.
13. Pommier, B.; Danascimento, S.; Dumont, S.; Bellier, B.; Million, E.; Garbay, C.; Roques, B. P.; Noble, F. *J. Neurochem.* **1999**, *73*, 281.
14. Sasaki, N. A.; Funakoshi, S.; Potier, P.; Morgat, J. L.; Genet, R.; Gacel, G.; Charpentier, B.; Roques, B. P. *J. Labelled Compd. Radiopharm.* **1985**, *22*, 1123.
15. Charpentier, B.; Roques, B. P.; Roy, J.; Morgat, J. L. *J. Labelled Compd. Radiopharm.* **1989**, *27*, 387.
16. L'abbé, G.; Leurs, S.; Dehaen, W. *Tetrahedron* **1993**, *49*, 4439.
17. Osby, J.; Martin, M. G.; Ganem, B. *Tetrahedron Lett.* **1984**, *25*, 2093.
18. Analytical data on BBL454: RMN ^{1}H (DMSO-d_6 + TFA) (δ ppm/HMDS; two rotamers due to the NMeNle residue): 0.6–1.7 (m, $-(CH_2)_3CH_3$ Nle and $CONHCH_2-(\underline{CH_2})_4-\underline{H}$) 2.38–3.1 (m, 10H, $CO\underline{CH_2}CONH\underline{CH_2}(CH_2)_4-$, $CH_2\beta$ Trp, $CH_2\beta$ Asp, $CH_2\beta$ Phe), 2.77 and 3.03 (s, 3H, NCH_3 Nle), 4.19 (m, α Nle), 4.72 (m, α Nle), 4.31 (m, 1H, α Phe), 4.40 and 4.48 (m, 1H, α Asp), 4.90 (m, 1H, α Trp), 6.85–7.16 (m) and 7.22 (d) and 7.45 (m) and 7.75 (d), (13H, 5 Ar Trp + 5 Ar Phe + amide NH Phe + $CONH_2$ Phe), 7.78 (d) and 7.88 (d) (1H, $N\underline{H}(CH_2)_5-H$) 7.90 (d) and 8.13 (d) (1H, amide NH Asp), 8.41 (d) and 8.83 (d) (1H, amide NH Trp) 10.68 and 10.78 (s, 1H, indole NH). **HPLC**: $t_R = 23.4$ min [gradient 10–90% B in 30 min (solvent systems A: H_2O + 0.1% TFA, B: 70% acetonitrile, 30% H_2O, 0.09% TFA), column: C18 Vydac (5 μ, 4.6 × 150 mm), 1 mL/min, λ = 210 nm].
19. Gigoux, V.; Escrieut, C.; Fehrentz, J. A.; Poirot, S.; Maigret, B.; Moroder, L.; Gully, D.; Martinez, J.; Vaysse, N.; Fourmy, D. *J. Biol. Chem.* **1999**, *274*, 20457.
20. Affinities for CCK_1 and CCK_2 receptors were determined respectively on guinea-pig pancreatic membranes and on membranes of CHO cells stably transfected with the rat CCK_2 receptor, by competition with 1 nM [^{3}H]pCCK-8. Results are the mean of at least three separate experiments, each performed in triplicate.