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STRUCTURE-BASED DESIGN AND PHARMACOLOGICAL PROPERTIES OF POTENT SELECTIVE AND SYSTEMICALLY ACTIVE CCK-B PEPTIDOMIMETICS

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Abstract : Selective CCK-A and CCK-B agonists and antagonists, having all the criteria required to be administered systemically have been developed. pBC 264, a selective CCK-B agonist has permitted the in vitro and in vivo characterization of brain receptors, and has been used to establish the involvement of CCK-B receptors in anxiety-related behaviour.

The octapeptide cholecystokinin (CCK_8) is abundant in both the brain and the gastrointestinal system where it interacts with a nanomolar affinity with at least two types of binding sites designated CCK-A and CCK-B receptors. CCK-B receptors are widely distributed in various areas of the brain whereas CCK-A receptors are found in only a few subcortical nuclei [1]. CCK-B sites resemble the gastrin receptor, which has been recently cloned [2].

Peripheral administration of low doses of CCK-B antagonists has been shown to produce anxiolytic effects [3] and potentiate morphine-induced analgesia [4]. Likewise panic attacks or severe anxiogenic syndrome were induced by peripheral administration of low doses of CCK_4 to healthy volunteers and to patients with panic disorders [5]. However, the respective role of central and/or peripheral receptors in these responses remains to be firmly established. An answer to these questions required agonists and antagonists selective for CCK-A and CCK-B receptors, which are capable of crossing the blood brain barrier.

Design of CCK_8 derived peptidomimetics with improved bioavailability :

The design of CCK agonists was based on the modification of BDNL ($Boc(Nle^{28,31})CCK_{27-33}$) a mixed agonist as potent as CCK_8. The peptide bonds were modified in order to improve their resistance to degradation. Retroinversion of the Nle-Gly bond and N-methylation of the (Nle^{31}) residue led to BC 264, a highly peptidase resistant compound with a high affinity and selectivity for CCK-B binding sites. BC 264 is a 50 times more potent CCK-B agonist than CCK_8 in electrophysiological and pharmacological assays [6]. Incorporation in BC 264 of the novel amino acid $Phe(pCH_2SO_3H)$ in place of $Tyr(SO_3H)$ by solid phase synthesis, led to a compound, RB 205, which retains all the properties of BC 264 and moreover fulfill all the required criteria for oral administration.

CCK-B versus CCK-A receptor recognition : criteria for agonist versus antagonists properties :

Binding characteristics and agonistic properties can be modulated by modifying the side chains of CCK_8 and CCK_4 related peptides. Introduction of an (Orn^{31}) residue in place of (Nle^{31}) in CCK_8-derived peptides led to full agonists. When the amine function in the side chain of (Orn^{31}) was protected by a benzyloxycarbonyl, the resulting peptides were mixed antagonists [7].

Introduction of (Phe^{31}) in place of (Nle^{31}) in BDNL led to an increase in CCK-B selectivity, since Boc(Nle^{28}, Phe^{31})CCK_{27-33} is about 60-fold more potent towards CCK-B than towards CCK-A receptors. Furthermore, incorporation of 1Nal-NH_2(1-naphthylalanine amide) in BDNL did not change the binding properties. When introduced in CCK_4 these modifications led to selective CCK-B agonists such as Boc-Trp-Phg-Asp-1Nal-NH_2 with a good affinity (K_I = 14 nM) and selectivity (78 fold) for CCK-B receptors. The apparent affinities of these compounds in guinea-pig are reported in Table 1. Boc-Trp-Phg-Asp-1Nal-NH_2 is a full agonist in electrophysiological studies on hippocampal neurons, an effect related be to CCK-B receptor activation [8]. Interestingly, incorporation of 1Nal-$N(CH_3)_2$ in this tetrapeptide led to a CCK-B selective antagonist in electrophysiological assay, with a good affinity for CCK-B receptors in the guinea-pig (K_I = 39 nM) and in the rat (K_I = 51 nM), only 5 times lower than the Merck CCK-B antagonist L365,260 (K_I = 11 nM). All these pseudopeptides proved to be highly resistant to peptidases, probably due to the presence of unatural residues. These CCK_4 related peptides could be used in defining the structural characteristics necessary for the recognition of the agonist and antagonist states of CCK-A and CCK-B receptors.

Table 1 : Apparent affinities of CCK_8 and CCK_4 derived peptidomimetics on the binding of [^{3}H]pCCK_8 (0.2 nM) to brain and pancreatic membranes of guinea-pig.

		K_I(nM)	
		CCK-B	CCK-A
Asp - Tyr(SO_3Na) - Met - Gly - Trp - Met - Asp - Phe - NH_2	(CCK_8)	0.28 ± 0 01	0.64 ± 0.04
Boc - Tyr(SO_3Na) - gNle - mGly - Trp - (N-Me)Nle - Asp - Phe - NH_2	(BC 264)	0.15 ± 0.02	78 ± 6
p - Tyr(SO_3Na) - gNle - mGly - Trp - (N-Me)Nle - Asp - Phe - NH_2	(pBC 264)	0.060 ± 0.008	62 ± 9
Boc - Phe(pCH_2SO_3Na) - gNle - mGly - Trp - (N-Me)Nle - Asp - Phe - NH_2	(RB 205)	0.46 ± 0.10	1100 ± 30
Boc - Trp - Phe - Asp - 1Nal - NH_2		71 ± 8	2400 ± 200
Boc - Trp - Phg - Asp - 1Nal - NH_2		14 ± 1	1100 ± 300
Boc - Trp - Phg - Asp - 1Nal - $N(CH_3)_2$		39 ± 1	1000 ± 400

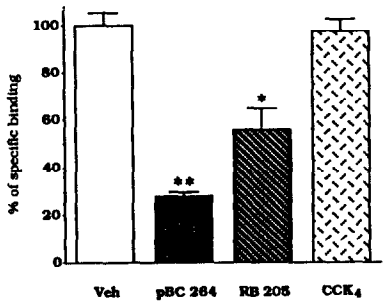
CCK-B receptor heterogeneity :

An analog of BC 264 with a tritiated propionyl protecting group in place of the butyloxycarbonyl group [^{3}H]pBC 264 (98 Ci/mmol) was synthesized, since the non tritiated analog was shown to be highly potent and selective for guinea-pig CCK-B receptors (K_I CCK-A : 62 nM; K_I CCK-B : 0.06 nM, selectivity ratio : 1038). [^{3}H]pBC 264 interacts apparently with a similar high affinity (0.2 nM) with one class of binding sites in mouse, rat, guinea-pig, cat and human brain. In guinea-pig and mouse brain, specific [^{3}H]pBC 264 binding was not affected by NaCl and guanylnucleotides, while in rat brain, the affinity of the tritiated probe was significantly decreased by guanylnucleotides or by alkaline treatment, suggesting that a proportion of CCK-B receptors are linked with G -proteins. Moreover, the relative potencies of various agonists and antagonists differed among species [9]. Competition experiments performed with some of these analogs were significantly better when fitted by a two-site than a one-site model. The proportion of high affinity sites was estimated to be around 10-15% in rat brain and around 50-60% in guinea-pig brain. These results suggest the presence of different types or subtypes, or different states of the same receptor, in various proportions in guinea-pig and rat brain.

In vivo binding of [^{3}H]pBC 264 :

In vivo binding experiments were performed after i.c.v. injection of 10 pmol [^{3}H]pBC264 as described [10]. I.v. administration of 20 mg/kg pBC 264 and RB 205 prior to i.c.v. injection of [^{3}H]pBC 264 inhibited the specific binding of the tritiated probe by about 72%, and 44% respectively, showing the ability of these probes to enter the brain. In contrast peripheral administration of CCK_4, was unable to modify [^{3}H]pBC 264 binding (Fig. 1).

Figure 1 : Comparison of the inhibition of the "in vivo" binding of 10 pmol [^{3}H]pBC 264, by pBC 264, RB 205 and CCK_4 administered i.v. at 20mg/kg



* p<0.05, ** p<0.001 as compared to controls (Student's t test).

As expected, the CCK-A antagonist (L364,718) did not inhibit [^{3}H]pBC 264 binding, and the CCK-B antagonist (L 365,260) produced a weak inhibition, 20% at the highest dose used (40 mg/kg i.p.) (data not shown). These results suggest that the anxiolytic properties induced by L 365,260 and the CCK_4-induced-panic attacks could involve an initial stimulation of peripheral CCK-B binding sites or the activation of high affinity CCK-B binding subtype [10]. Endogenous enkephalins, protected from enzymatic degradation by inhibitors such as RB 101 or kelatorphan, increased the release of CCK_8, via delta opioid receptor activation, leading to an inhibition of the specific [^{3}H]pBC 264 binding [11]. This suggests a control between CCKergic and enkephalinergic pathways, which could be a homeostatic mechanism to re-establish the nociceptive threshold after stimulation of opioid receptors.

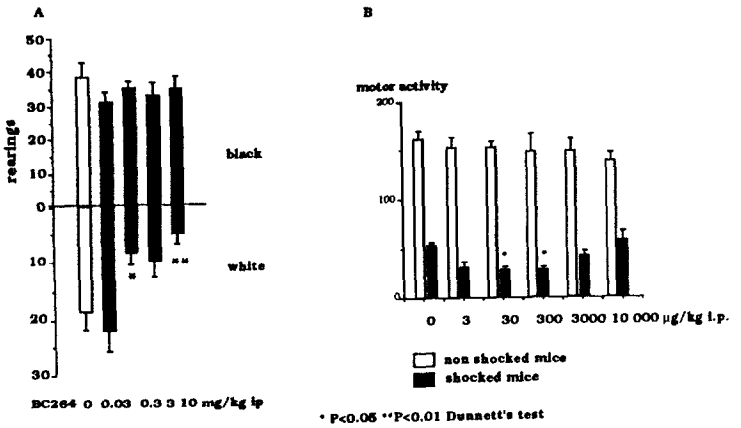
Effect of BC 264 in anxiety :

The possible involvement of CCK-B receptors in anxiety has been studied 30 min after i.p. injection in mice of the selective CCK-B agonist BC 264 at doses of 0.03, 0.3, 3 and 10 mg/kg in the black and white test box [12]. This behavioural paradigm using exploration of a novel environment is based on findings that highly illuminated open fields have aversive properties which inhibit rodent exploration (white box). Doses of 0.3 and 10 mg/kg of BC 264 were shown to significantly decrease rearings (Fig. 2) in the white box. Furthermore, locomotion and time spent were also significantly decreased at 10 mg/kg i.p. In contrast, the behaviour of mice was not changed in the black part of the box. The changes produced by BC 264 seem therefore to be related to anxiogenic-like effects.

The effect of BC 264 (3 to 10,000 µg/kg i.p., injected 30 min before experiment) on the conditioned suppression of motility has also been investigated. As previously described [13] mice were

placed in a transparent rectangular cage where they received or not (control group) electric foot shocks the day before. Motility changes were observed by counting the number of squares crossed and the number of rearing for 6 min. At the doses of 30 and 300 µg/kg, BC 264 was found to significantly accentuate the suppression of motility in stressed mice without changing the motility in non shocked-mice. These results also confirm that CCK, through CCK-B receptors, could potentiate anxiety-related behaviour. The bell shaped dose response curve, often observed with CCK, could be relevant to the existence of CCK receptor subtypes [9].

Figure 2 : Effect of BC 264 in mice, A : black and white box, B : conditioned suppression of motility.



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