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Peptoid CCK receptor antagonists: pharmacological evaluation of CCK_A, CCK_B and mixed $CCK_{A/B}$ receptor antagonists

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Abstract

Several novel cholecystokinin (CCK) receptor ligands with differing degrees of receptor selectivity were characterised in both in vitro and in vivo models. In radioligand binding assays, the dipeptoid PD 135666 ((benzenebutanoic acid, β-[[3-(1*H*-indol-3-yl)-2-methyl-1-oxo-2-[[(tricyclo[3.3.1.1^{3,7}]dec-2-yloxy)carbonyl]amino]propyl]amino],-[*R*-(*R**,*S**)]) selectively inhibited [^{125}I]Bolton Hunter CCK-8 binding to CCK_B receptors in mouse cerebral cortex (CCK_B IC_{50} = 0.1 nM) but was weaker as an inhibitor of CCK_A receptor binding in the rat pancreas (IC_{50} = 26 nM). In contrast, its enantiomer PD 140548 ((benzenebutanoic acid, β-[[3-(1*H*-indol-3-yl)-2-methyl-1-oxo-2-[[(tricyclo[3.3.1.1^{3,7}]dec-2-yloxy)carbonyl]amino]propyl] amino],-[*S*-(*R**,*S**)) displayed the reverse selectivity (CCK_A IC_{50} = 2.8 nM, CCK_B IC_{50} = 260 nM). PD 142898 ({benzenebutanic acid, β-[[3-(1*H*-indol-3-yl)-2-methyl-2-[[[(2-methylcyclohexyl)oxy]carbonyl]amino]-1-oxopropyl]amino]-,[1*S*-[1α[*S**(*R**)],2β]]) possessed nanomolar affinity for both receptor subtypes (CCK_B IC_{50} = 4.2 nM, CCK_A IC_{50} = 3.8 nM) whereas its corresponding enantiomer PD 142896 ({benzenebutanic acid, β-[[3-(1*H*-indol-3-yl)-2-methyl-2-[[[(2-methylcyclohexyl)oxy]carbonyl]amino]-1-oxopropyl]amino]-, [1*R*-[1α[*S**(*R**)],2β]]) displayed 147-fold selectivity for the CCK_A receptor (CCK_A IC_{50} = 7.9 nM, CCK_B IC_{50} = 1160 nM). The pyrazolidinone PD 141479 (*trans*-5-(2-chlorophenyl)-3-oxo-4-phenyl-*N*-[4-(trifluoromethyl)phenyl]-1-pyrazolidinecarboxamide) was found to interact selectively with the CCK_B receptor (CCK_B IC_{50} = 36 nM, CCK_A IC_{50} = 1100 nM). PD 140548, PD 142896, PD 135666 and PD 142898 antagonised the CCK_A receptor-mediated contraction of guinea pig gall bladder with respective pA_2 values of 7.2, 7.4, 6.6 and 8.5. In the rat elevated X-maze, PD 135666 and PD 141479, together with the mixed $CCK_{A/B}$ receptor antagonist PD 142898 produced anxiolytic effects with respective minimum effective doses (MEDs) of 0.01, 0.001 and 0.01 mg/kg s.c. Furthermore, the selective CCK_B receptor antagonist CI-988 (0.01–1 mg/kg) and PD 142898 (0.001–0.1 mg/kg), dose dependently induced behavioural changes suggestive of anxiolysis in the marmoset human threat test with respective MED values of < 0.01 and < 0.001 mg/kg s.c. In contrast, compounds with the CCK_A selective profile were either inactive in the two behavioural models or showed activity only at doses of 1 mg/kg and above. These data suggest that the anxiolytic effects of CCK receptor antagonists parallel their affinity for the CCK_B rather than the CCK_A receptor.

Keywords: CCK_A receptor; CCK_B receptor; $CCK_{A/B}$ receptor antagonist, mixed; Radioligand binding; Gall bladder, guinea pig; Anxiolytic

1. Introduction

Cholecystokinin (CCK) is a peptide that was originally discovered in the gastrointestinal tract (Ivy and Oldberg, 1928). It has been shown to be present as the sulphated octapeptide (CCK-8S) in high concentrations in various regions of the brain (Vanderhaeghen et al., 1975; Dockray, 1976; Savasta et al., 1988; Innis et al., 1979; Rehfeld, 1978), where it may serve as a neurotransmitter/modulator (Vanderhaeghen et al., 1975). Receptors for CCK have been divided into two subtypes (Innis and Snyder, 1980; Moran et al., 1986). (i) The CCK_A receptor, that is prominently found in some peripheral tissues but is also present in discrete brain regions (Innis and Snyder, 1980; Hill et al., 1987a,b). The sulphated form of the *C*-terminal octapeptide (CCK-8S) shows higher affinity for the CCK_A receptor than other peptides of the same series, such as the desulphated form of CCK-8 and the tetrapeptide

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(CCK-4). The actions mediated by the CCK_A receptor are selectively blocked by the antagonist devazepide (formerly MK-329; Chang and Lotti, 1986). (ii) The CCK_B receptor is found throughout the brain (Saito et al., 1980; Gaudreau et al., 1983; Van Dijk et al., 1984; Hill et al., 1987a; Boden and Hill, 1988; Bohme et al., 1991). It is characterised by high affinity for the peptide agonists such as pentagastrin and CCK-4, and the novel antagonists CI-988 (formerly PD 134308; Hughes et al., 1990), L-365,260 (Lotti and Chang, 1989) and a pyrazolidinone derivative LY262691 (Howbert et al., 1991).

Recent studies have indicated that CCK may have a modulatory role in anxiety, acting via interactions with the CCK_B receptor. Thus, it has been demonstrated that activation of central CCK_B receptors with the selective agonists pentagastrin and CCK-8 induces an anxiogenic-like action, whilst the selective antagonists of this receptor produce anxiolytic-like effects in a wide range of animal models of anxiety (Hughes et al., 1990; Costall et al., 1991; Singh et al., 1991a,b; Rataud et al., 1991).

However, the role of brain CCK_A receptors in anxiety remains unclear. In our and other hands (Dauge et al., 1989; Singh et al., 1991a,b; Rataud et al., 1991) devazepide, a highly selective CCK_A receptor antagonist, showed no anxiolytic-like effects at doses that would selectively antagonise CCK_A receptors (Dauge et al., 1989). However, in two preliminary studies devazepide did appear to produce anxiolytic-like effects at low doses in the mouse light/dark box and the rat elevated X-maze tests of anxiety (Hendrie and Dourish, 1990; Ravard et al., 1990).

Previously it has been reported that selective CCK_B receptor antagonists of different chemical classes, possess potent anxiolytic-like action in several animal models of anxiety (Hughes et al., 1990; Rataud et al., 1991; Singh et al., 1991a,b; Chopin and Briley, 1993). We now report that novel and selective CCK_A receptor antagonists selected from the same dipeptoid chemical series as the selective CCK_B receptor antagonist CI-988 (Hughes et al., 1990), at low doses do not show such anxiolytic-like actions. In contrast, the corresponding enantiomers of these compounds and a pyrazolidinone derivative that are selective CCK_B receptor antagonists induce potent anxiolytic-like action. Details of the chemical syntheses has been described elsewhere (Boden et al., 1993).

2. Materials and methods

2.1. Radioligand binding studies

CCK_A and CCK_B receptor binding assays were performed on male rat pancreas and male mouse cerebral cortex respectively using [^{125}I]Bolton Hunter CCK-(26–33) (35 pM) as previously described (Hughes et al., 1990). Non-specific binding was estimated by adding 1 μM CCK-8S.

2.2. Guinea-pig gall bladder preparation

Adult male Dunkin-Hartley guinea-pigs (330–400 g, Bantin and Kingman Universal, UK) had free access to food and water. Animals were killed by cervical dislocation, and the gallbladder rapidly removed and opened along the greater curvature. Strips of muscle (2×25 mm) were mounted in siliconised 3 ml organ baths containing a modified Krebs-Henseleit solution of the following composition (mM): NaCl 118, KCl 5.9, $MgSO_4$ 1.2, $CaCl_2$ 2.5, NaH_2PO_4 1.2, $NaHCO_3$ 25.5 and glucose 11.1. The solution was maintained at 37°C and was gassed continuously with a mixture of 95% O_2-5% CO_2. Isometric contractile responses were measured with Grass FT.03 force-displacement transducers and recorded on Graphtec Linearcorders (MarK VII).

Tissues were placed under 1 g tension and allowed to equilibrate for 30 min after which they were contracted with a submaximal dose of CCK-8S (10 nM). Using cumulative dosing response curves were established for CCK-8S, with all agents added in volumes not exceeding 10 μl. For antagonist studies tissues were exposed to antagonists for at least 15 min before re-exposure to agonist.

Contractile responses to CCK-8S were expressed as absolute changes in tension and then transformed to a percentage of the maximal response achieved for the one preparation. Responses in the presence of antagonists were expressed as a percentage of the control maximum response obtained in the same tissue preparation. In most cases agonist log(concentration)-response curves were constructed in the absence and presence of a range of concentrations of CCK receptor antagonists to obtain values for dose ratio (at EC_{50}), and estimates of antagonist affinity derived by regression analysis of the plot of log (dose ratio − 1) against log[antagonist] to obtain values for pA_2 and slope. Where the slope did not differ significantly from one, Schild regression analysis was repeated with the slope constrained to unity to obtain estimates of affinity as pK_B. Where the affinity of the antagonist was not sufficiently high, or stocks of compound were limited, the equilibrium dissociation constant (apparent K_B) was obtained using the equation: $pK_B = -\log \{[\text{antagonist}]/(\text{dose ratio} - 1)\}$.

2.3. Behavioural studies

Rat elevated X-maze

Male Hooded Lister rats (200–250 g) were obtained from Olac (Bicester, UK) and were housed in groups of

six under a 12 h light/dark cycle (lights on at 07:00 h) with food and water ad libitum. All behavioural studies were carried out between 10:00 h and 17:00 h. The effect of compounds on the elevated X-maze was measured as previously described (Singh et al., 1991b). Briefly, animals were placed on the centre of the X-maze facing one of the open arms. The entries and time spent on the end half sections of the open arms, and total entries were measured during the 5 min test period.

Marmoset human threat test

Ten Common Marmosets (Callithrix Jacchus) weighing between 280 and 360 g, bred at Manchester University Medical School (Manchester, UK), were housed in pairs under a 12 h light/dark cycle (lights on at 7:00 h) in a room maintained at 24–26°C with 55% humidity. Animals had free access to water and were fed mazuri pellets and fruit early morning and late afternoon. All experiments were carried out in home cages which were divided into two equal halves on the test day. Marmosets were habituated to being separated from their partner between 11:00 and 15:00 h.

The total number of body postures exhibited by the animal towards the threat stimulus (a human standing approx. 0.5 m away from the marmoset cage and staring into the eyes of the marmoset) was recorded during the 2 min test period. The body postures scored were slit stares, tail postures, scent marking of the cage/perches, piloerection, retreats and arching of the back. Each animal was exposed to the threat stimulus twice on the test day before and after drug treatment. All drug treatments were carried out s.c. at least 2 h after the first (control) threat. Results are expressed as percent decrease in postures (control threat compared to the post drug threat). The pretreatment time for each compound was 40 min. These experiments were carried out at University of Manchester Medical School, Department of Structural and Cell Biology, Manchester, UK between 11:00 an 15:00 h.

2.4. Drugs

The following compounds were synthesised at Parke-Davis Neuroscience Research Centre, Cambridge, UK: PD 135666 (Benzenebutanoic acid, β-[[3-(1*H*-indol-3-yl)-2-methyl-1-oxo-2-[[(tricyclo[3.3.1.1^{3,7}]-dec-2-yloxy)carbonyl]amino]propyl]amino],-[*R*-(*R**,*S**)], PD 140548 (benzenebutanoic acid, β-[[3-(1*H*-indol-3-yl)-2-methyl-1-oxo-2-[[(tricyclo[3.3.1.1^{3,7}]-dec - 2 - yloxy)carbonyl]amino]propyl] amino], - [*S* - (*R**,*S**)], PD 142896 {benzenebutanic acid, β-[[3-(1*H*-indol-3-yl)-2-methyl-2-[[[(2-methylcyclohexyl)oxy]-carbonyl]amino] - 1 - oxopropyl]amino] -, [1*R* - [1α[*S**(*R**)],2β]] and PD 142898 {benzenebutanic acid, β-[[3-(1*H*-indol-3-yl)-2-methyl-2-[[[(2-methylcyclohexyl)oxy]carbonyl]amino]-1-oxopropyl]amino]-,[1*S*-[1α-[*S**(*R**)],2β]]. The pyrazolidinone derivative PD 141479 (*trans*-5-(2-chlorophenyl)-3-oxo-4-phenyl-*N*-[4-(trifluoromethyl)phenyl]-1-pyrazolidinecarboxamide; Howbert et al., 1991) was synthesised at Parke-Davies, Ann Arbor, Michigan, USA. All other drugs and reagents were obtained from commercial sources.

For behavioural studies, all compounds were dissolved in 0.9% w/v NaCl except PD 141479 which was suspended in 1.0% w/v carboxymethylcellulose with the aid of ultrasonification. Drugs were administered s.c. 40 min before test in a volume of 1 ml/kg.

3. Results

3.1. Receptor binding studies

Compounds were tested as inhibitors of [^{125}I]Bolton Hunter CCK-8 binding to CCK_A and CCK_B recognition sites prepared from rat pancreas and mouse cerebral cortex, respectively. PD 135666 and PD 141479

Table 1
Inhibition of [^{125}I]Bolton Hunter CCK-8 binding to membranes prepared from mouse cerebral cortex (CCK_B) or rat pancreas (CCK_A)

Compound	IC_{50} (nM)				CCK_A/CCK_B
	Rat pancreas CCK_A		Mouse cortex CCK_B		
CCK-8S *	0.1	(0.08–0.2)	0.8	(0.5–0.9)	0.33
PD 135666	26	(18–36)	0.15	(0.09–0.2)	170
PD 140548	2.8	(1.4–5.1)	260	(210–290)	0.01
PD 141479	1100	(750–1600)	36	(27–42)	31
PD 142896	7.9	(6.5–9.4)	1160	(823–1680)	0.007
PD 142898	3.8	(2.1–5.4)	4.2	(3.8–4.71)	0.9
Devazepide *	0.1	(0.03–0.2)	31	(18–43)	0.003
CI-988 *	4300	(120–8500)	1.7	(1.3–2.7)	2500

IC_{50} represents the concentration (nM) producing half maximal inhibition of specific [^{125}I]Bolton Hunter CCK-8 binding (35 pM). The values shown represent the geometric mean and the range (in parentheses) from at least three separate experiments. * For comparison data taken from Horwell et al. (1991).

produced a concentration-dependent inhibition of radioligand binding to CCK_B recognition sites with IC_{50} values of 0.15 and 36 nM, respectively (Table 1). In contrast, the IC_{50} values for PD 135666 and PD 141479 for displacing the radioligand from CCK_A sites were 26 and 1100 nM, respectively, indicating both compounds were selective for the CCK_B receptor (Table 1). PD 140548 which is the corresponding (*R,S*) enantiomer of PD 135666 showed nanomolar affinity for the CCK_A binding sites ($IC_{50} = 2.8$ nM) and was almost 100-fold weaker at displacing the radioligand from the CCK_B sites ($IC_{50} = 260$ nM; Table 1).

PD 142898 displaced the radioligand from both CCK receptor subtypes with similar affinity (CCK_B $IC_{50} = 4.2$ nM; CCK_A $IC_{50} = 3.8$ nM; Table 1). In contrast, PD 142896 which is the corresponding (*R,R,S,R*) enantiomer of PD 142898 showed 100-fold higher affinity for the CCK_A binding site (CCK_A $IC_{50} = 7.9$ nM; CCK_B $IC_{50} = 1160$ nM; Table 1).

3.2. Antagonism of CCK-8S induced contractions of guinea pig gall bladder

As has been noted by numerous investigators, cumulative addition of CCK-8S produced concentration-related contraction of the gall bladder over a wide range (threshold ≤ 0.1 nM, maximum ≥ 1 μM; pEC_{50} 8.08 $\pm$ 0.07, $n = 28$). Consistent with mediation of contraction by a CCK receptor of the A-type, devazepide antagonised the response to CCK-8S in an apparently competitive manner and with high affinity (apparent pK_B 10.04 $\pm$ 0.17, $n = 3$). The non-selective dipeptoid PD 142898 was a competitive antagonist (pA_2 8.5, slope 0.92) with functional affinity (pK_B 8.30; 95% c.l. 8.07, 8.52; d.f. 9) in line with binding affinity; this was also true for the pyrazolidinone PD 141479 (apparent pK_B 6.30 $\pm$ 0.13, $n = 4$). While the remaining dipeptoids PD 140548, 142896 and 135666 all appeared to be competitive antagonists at the CCK_A receptor in the gall bladder (respective pA_2 values: 7.2, slope 0.93; 7.4, slope 0.81; 6.6, slope 1.1), affinities were 8-to 30-fold lower (values for K_B 75.8, 74.1 and 204.2 nM, respectively) than from binding in rat pancreas. None of the dipeptoids showed any ability to contract the gall bladder at concentrations up to 3 μM.

3.3. Effect of CCK receptor antagonists in the rat elevated X-maze

The s.c. administration of PD 135666, PD 141479 and PD 142898, 40 min before test, increased dose dependently the percent time spent on and entries made onto the end half sections of the open arms with minimum effective doses (MED) of 0.01, 0.001 and 0.01 mg/kg, respectively (Fig. 1). However, doses higher than 0.1 mg/kg of PD 135666 and PD 141479 pro-

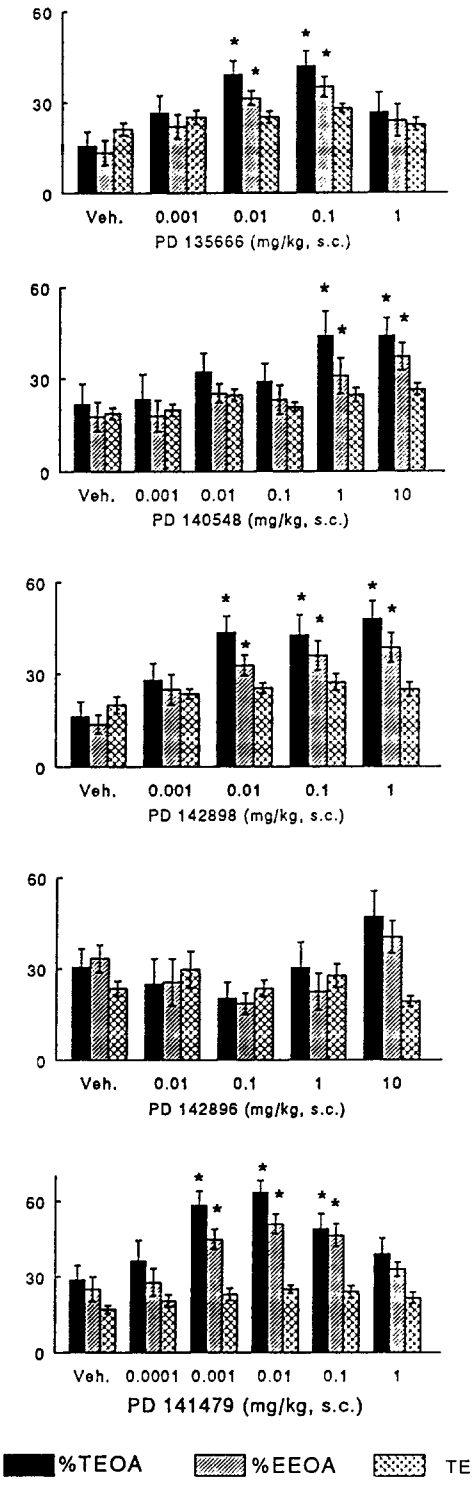

Fig. 1. Effect of CCK receptor antagonists in the rat elevated X-maze. Test compounds were administered s.c. 40 min before test. The percent time spent (black columns) and entries (hatched columns) made on to the end half sections of the open arms, and the total number of entries (cross-hatched columns) were measured during the 5 min test period. Results are shown as the mean (vertical bars represent $\pm$S.E.M.) of ten animals per group. Control animals received the vehicle (Veh.). * Significantly different from controls, $P < 0.05$ (ANOVA followed by Dunnett's *t*-test).

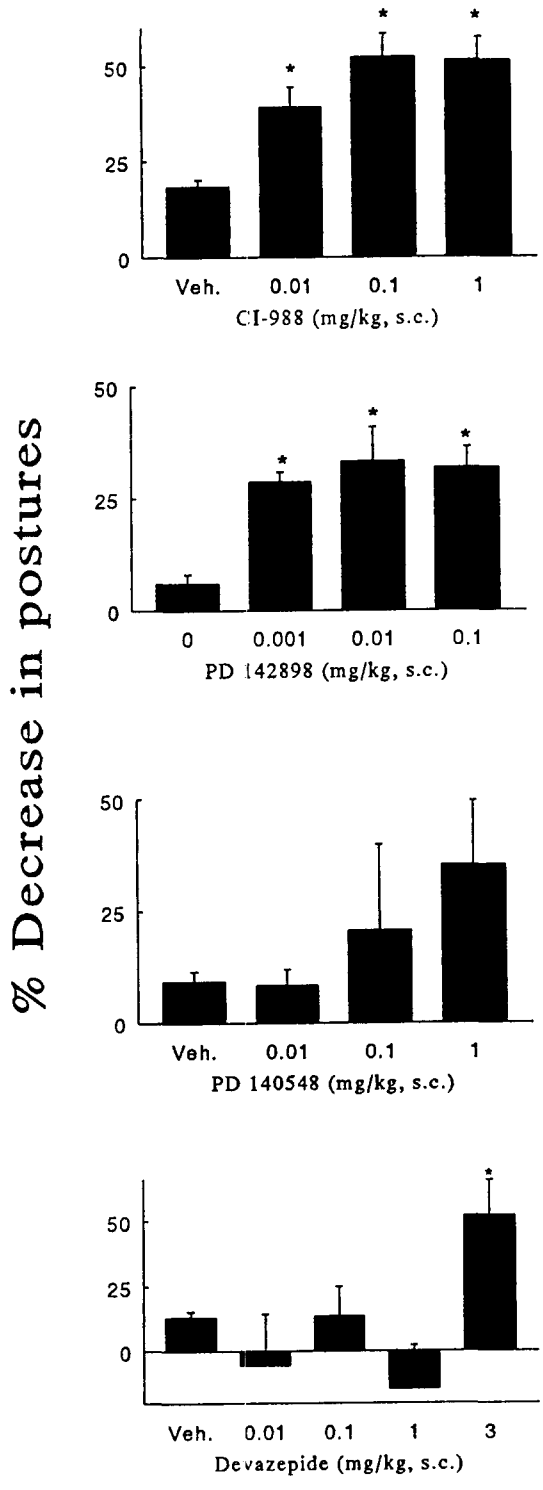

Fig. 2. Effect of CI-988, PD 140548, PD 142898 and devazepide in the marmoset human threat test. Each animal was exposed to a 2 min threat stimulus before (control threat) and after dosing (post dose threat). Results are expressed as percent decrease in postures (control threat compared to the post drug threat for each animal). Test compounds were administered s.c. 40 min before test. Results are shown as percent decrease in postures (vertical bars represent ± S.E.M.) of five to six animals per group. * Significantly different from mean day controls, $P < 0.05$ (ANOVA followed by Dunnett's t-test).

duced a decrease in this anxiolytic-like action (Fig. 1). Similar administration of PD 140548 produced an anxiolytic-like action only at high doses of 1 and 10 mg/kg (Fig. 1). PD 142896 was found to be inactive up to 10 mg/kg (Fig. 1). None of the compounds tested affected total entries into the open and enclosed arms indicating lack of effect on motor activity (Fig. 1).

3.4. Effect of CCK receptor antagonists in the marmoset human threat test

The s.c. administration of CI-988 (0.01–1 mg/kg) or PD 142898 (0.001–0.1 mg/kg), 40 min before test decreased dose dependently the number of postures exhibited by marmosets in a response to the human threat (Fig. 2). CI-988 and PD 142898 induced these changes considered to be indicative of anxiolytic-like action, with respective MEDs of < 0.01 and < 0.001 mg/kg. In contrast, similar administration of devazepide (0.01–3.0 mg/kg) or PD 140548 (0.01–1.0 mg/kg) failed to reduce the number of postures, suggesting lack of anxiolytic-like action over the lower dose range (Fig. 2). However, a reduction was observed at the very high dose of 3 mg/kg devazepide (Fig. 2).

4. Discussion

In the present study we have described and characterised novel and selective CCK_A, CCK_B and a mixed $CCK_{A/B}$ receptor antagonists. The chemically different CCK_B receptor ligands pyrazolidinone PD 141479 and the dipeptoid PD 135666 were between 31 and 170 times selective for CCK_B versus CCK_A receptors with PD 135666 showing nanomolar affinity for the [^{125}I]Bolton Hunter CCK labelled CCK_B receptors in the mouse brain. In contrast, PD 140548 the corresponding (*S*,*R*)-enantiomer of PD 135666 was 100 times selective for the CCK_A versus the CCK_B receptor and displaced binding of [^{125}I]Bolton Hunter CCK to CCK_A receptors in rat pancreas with nanomolar affinity. Although PD 142898 showed no selectivity between the two CCK receptors, its corresponding (*R*,*R*,*S*,*R*)-enantiomer PD 142896 was 147 times more selective for the CCK_A receptor.

The results from the functional studies presented here show that the compounds described are competitive antagonists at CCK receptors and do not possess any intrinsic efficacy. Previous studies from our laboratory have shown that the excitatory effects of pentagastrin mediated by CCK_B receptors in the rat VMH were powerfully antagonised by PD 135666 and PD 141479 (Boden et al., 1994). In the present study, PD 140548 and PD 142896 antagonised the CCK_A receptor-mediated contractions of guinea pig gall bladder. However, the affinity of the two CCK_A receptor antagonists in

the latter functional assay were lower than that would be expected from their binding to rat pancreatic CCK_A recognition sites. The reason for this apparent anomaly is unclear but it may be due to a species difference. Nevertheless, when compared with the corresponding enantiomers, both PD 140548 and PD 142896 were 100-fold weaker at antagonising the CCK_B receptor in the rat VMH (Boden et al., 1994). PD 142898 with nanomolar $CCK_{A/B}$ binding affinities, antagonised responses mediated by both CCK_A and CCK_B receptors (Boden et al., 1994) indicating that this compound is a high affinity but mixed $CCK_{A/B}$ receptor antagonist.

In the rat elevated X-maze, the selective CCK_B receptor antagonists PD 135666 and PD 141479, induced anxiolytic-like action. The present results are consistent with a previous report (Hughes et al., 1990) showing that CI-988 can induce anxiolysis in the marmoset human threat test. Taken together these results are consistent with previous studies showing that other selective CCK_B receptor antagonists possess anxiolytic activity in experimental models of anxiety (Hughes et al., 1990; Costall et al., 1991; Singh et al., 1991a,b; Rataud et al., 1991; Chopin and Briley, 1993). The reason for the decrease in the effect of PD 135666 and PD 141479 at higher doses in the present study is unclear. However, due to the lack of effect of these compounds on locomotor activity, it is unlikely to be due to a sedative action. It has been reported that CCK_B receptor agonists produce anxiogenic-like action in the rat elevated X-maze (Singh et al., 1991a,b). Functional studies have shown that both PD 135666 and PD 141479 appear to be antagonists at the CCK_B receptor with no evidence of any agonist-like activity (Boden et al., 1994). Therefore, the lack of effect of these antagonists at higher doses is also unlikely to be due to an anxiogenic-like action mediated by the CCK_B receptor.

The results of this and our previous study do not support the involvement of CCK_A receptors in anxiety. The two CCK_A receptor antagonists PD 140548 and PD 142896 were 100–1000 times less potent in the elevated X-maze than their respective enantiomers which antagonise the CCK_B receptor with high affinity. Furthermore, devazepide and PD 140548 were either inactive or showed activity only at very high doses in the marmoset human threat test. Electrophysiological studies show that at high concentrations devazepide and PD 140548 can lose selectivity and act as CCK_B receptor antagonists. Therefore this antagonist effect at the CCK_B receptor may account for the activity in the two behavioural tests. These results indicate that at least in animal models, the CCK_A receptor plays little or no role in modulating anxiety. This is further supported by the finding that whilst the mixed $CCK_{A/B}$ receptor antagonist PD 142898 was active at low doses in the elevated X-maze and the marmoset human threat test it was no more effective than the selective CCK_B receptor antagonists.

The data presented here support and extend previous results indicating that CCK modulates anxiety-related behaviours in animals via interactions with the CCK_B receptor. It remains to be seen whether these series of compounds show efficacy in human generalised anxiety disorders. Recent studies have shown that selective CCK_B receptor antagonists show weaker activity than benzodiazepines in standard conflict tests (Powell and Barrett, 1991; Singh et al., 1991b; Dooley and Klamt, 1993). However, in tests that do not involve shock-induced conflict (e.g. elevated X-maze, social interaction, light/dark box, marmoset threat test), CCK_B receptor antagonists are as effective as the benzodiazepines. The reason for this difference is unknown but it is possible that shock-induced conflict tests measure either different forms of anxiety, involve a non-anxiety component, or involve a more severe form of anxiety. The emerging data from clinical anxiety studies with CCK_B receptor antagonists may provide valuable information regarding the relative usefulness of different animal models for predicting anxiolytic activity.

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