Evaluation of a series of novel CCK_B antagonists using a functional assay in the rat central nervous system

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1 Electrophysiological recordings from rat ventromedial hypothalamus (VMH) *in vitro* have been used to compare the effects of novel chemical entities on CCK_B receptor activation in the rat central nervous system.

2 Twenty compounds from three different chemical series were evaluated for their ability to reduce pentagastrin-induced increases in action potential firing rate.

3 All twenty compounds studied were found to be CCK_B antagonists, with equilibrium constants spanning a concentration-range of several orders of magnitude. The rank order for their ability to block pentagastrin responses correlated well with values obtained for their relative affinities for the mouse cortex CCK_B binding site.

4 It is concluded that the VMH preparation provides a good functional correlate to binding assays in the rodent central nervous system for a structurally diverse series of CCK_B antagonists.

Keywords: Novel CCK_B antagonists; hypothalamus; electrophysiology

Introduction

Cholecystokinin (CCK) is an important hormone, existing in both the mammalian gastrointestinal tract and central nervous system. In the latter CCK exists predominantly as the sulphated octapeptide (Dockray, 1976; Innis *et al.*, 1979), acting either at the gastrin/CCK_B receptor or at the more discretely localized CCK_A receptor to modify a wide variety of centrally-mediated behavioural events, including feeding (Gibbs & Smith, 1973; Dourish *et al.*, 1989), analgesia (Jurna & Zetler, 1981; Faris *et al.*, 1983; Watkins *et al.*, 1983) and anxiety (Bradwejn *et al.*, 1990). The relative importance of the two CCK receptor types in modulation of each of these behavioural states is unclear but the design and synthesis of highly selective antagonists for both CCK_A (Chang & Lotti, 1986) and CCK_B (Lotti & Chang, 1989; Hughes *et al.*, 1990) receptors has provided the opportunity to isolate the events following activation of a particular CCK receptor sub-type in the central nervous system.

Novel CCK_B antagonists, notable for their structural similarities, are now available. Non-peptide CCK_B antagonists have been derived from three different approaches. The gastrin/CCK_B antagonist L365,260 was produced following modification of the selective CCK_A receptor antagonist devazepide (Evans *et al.*, 1986; 1988). L365,260 displays a 100 fold greater affinity for the CCK_B receptor than for the CCK_A receptor (Lotti & Chang, 1989). Research at Lilly has produced a series of CCK_B antagonists following screening and structure-activity work based on early lead compounds culminating in the synthesis of LY-262691. This compound shows improved selectivity for the CCK_B receptor when compared to L365,260 (400 fold cf. 100 fold) although its affinity for the CCK_B receptor *per se* is lower (Bock, 1991). Horwell (1991) using a peptoid approach to modify the smallest active fragment of CCK, CCK-4, has produced CI-988. This compound has the highest CCK_B binding affinity when compared to the aforementioned compounds and is the most selective CCK_B receptor antagonist to date (Woodruff *et al.*, 1991) it is also a very effective anxiolytic in a number of animal models (Hughes *et al.*, 1990; Woodruff *et al.*, 1991) and enhances morphine-induced analgesia in rats (Weisenfeld-Hallen *et al.*, 1990).

At present, the number of functional assays for central CCK_B receptors is limited. The best characterized is the rat ventromedial hypothalamus (VMH) which possesses neur-

ones the spontaneous action potential firing rate of which is increased by CCK (Boden & Hill, 1988). A great majority (>90%) of VMH neurones are CCK-sensitive and the preparation has a high density of CCK_B receptors (Woodruff *et al.*, 1991). Using this model we have shown that peptoid CCK_B ligands are powerful antagonists of CCK-8s induced excitation (Hughes *et al.*, 1990) but the use of a non-selective agonist such as the sulphated C-terminal octapeptide can sometimes lead to activation of a mixture of both CCK_B and CCK_A receptors (Pinnock *et al.*, 1992), leading to possible under-estimation of functional activity. To overcome this difficulty in CCK_B antagonist studies we have used the selective agonist, pentagastrin, sometimes in combination with the selective CCK_A antagonist, devazepide, in an attempt to eliminate all possible interactions arising from activation of CCK_A receptors.

The aims of the present study were 2 fold. Firstly, to validate the use of an *in vitro* brain slice VMH preparation for quantitative pharmacological studies of CCK_B antagonists in the central nervous system. To this end the CCK_B antagonist activity of a number of novel chemical entities spanning a wide range of binding affinities from the same chemical series were investigated. Secondly, to compare the three chemically distinct CCK_B antagonist classes in the same paradigm at least one representative from each of the aforementioned drug design strategies was included in the present study.

Methods

Electrophysiology

Extracellular recordings were made from rat VMH neurones contained in coronal slices of rat midbrain. Hypothalamic slices were prepared from the brains of male Wistar rats (100–200 g) as described previously (Boden & Hill, 1988) and incubated in pre-gassed (95% O₂:5% CO₂) artificial cerebrospinal fluid of the following composition (mM): NaCl 124, KCl 5, CaCl₂ 2.4, NaH₂PO₄ 1.2, MgCl₂ 1.2, glucose 11, NaHCO₃ 25 at room temperature (21–23°C). One hour prior to recording, one slice was pinned to the Sylgard base of a Perspex chamber where it was submerged in ACSF and perfused by a gravity-fed perfusion system with ACSF at 37°C flowing at 4 ml min⁻¹. Drugs were applied directly

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dissolved in the perfusing medium, three-way taps being used to switch to a drug-containing ACSF. When included in the perfusing ACSF, devazepide((S)-N-(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1H-1,4-benzodiazepin-3-yl)-1H-indole-2-carboxamide) was added at a concentration of 10 nM for at least 20 min prior to recording. No detectable difference was found between shifts in pentagastrin dose-response curves using relatively low concentrations of CCK_B antagonists when devazepide was omitted from the ACSF but the CCK_A antagonist was used routinely at a concentration of 10 nM in studies assessing the actions of powerful CCK_B antagonists at high concentrations when the amounts of pentagastrin required to overcome the block might reach levels sufficient to activate CCK_A receptors. CCK_B antagonists were included in the perfusing solution for 30 min before construction of a second pentagastrin concentration-response curve in the presence of the antagonist. No change in the magnitude of the block produced by the antagonists was found when longer (up to 2 h) pre-incubation times were used. Data collection and analysis were performed with previously described methodology (Boden & Hill, 1987; Hughes *et al.*, 1990; Pinnock *et al.*, 1992). Final ratemeter histograms and dose-response curves were obtained by importing the files into a graphics and statistics software package (RS1, BBN software products, Cambridge, MA, U.S.A.). The same software was used to generate the correlation curve shown in Figure 1.

Receptor binding

Male mice were stunned and decapitated and the whole brain removed. The cerebral cortex was dissected free, weighed and homogenized in 10 volumes of ice-cold Tris buffer (pH 6.9 at 21°C) in a Polytron (setting 6 for 10–15 s). The homogenate was then centrifuged for 15 min at 20,000 g and the resulting pellet washed by re-suspension in Tris-HCl using a Polytron followed by re-centrifugation.

The final pellet was re-suspended in a standard assay buffer (10 mM HEPES, 130 mM NaCl, 4.7 mM KCl, 5 mM MgCl₂, 1 mM EGTA containing 0.25 mg ml⁻¹ bacitracin) at a tissue concentration of 2 mg of original wet weight ml⁻¹ of assay buffer.

For binding assays, aliquots (400 µl) of cortical membranes were incubated with a fixed concentration (35 pM) of [¹²⁵I]-Bolton-Hunter CCK-8 ([¹²⁵I]-CCK-8 specific activity, 2000 Ci mmol⁻¹) for 120 min at 21°C in the absence and presence of a range of concentrations (3 × 10⁻¹¹ to 1 × 10⁻⁶ M) of unlabelled test compound to give a final assay volume of 500 µl. Non-specific binding was that remaining in the presence of either 1 µM CCK-8s or CI-988. Non-specific binding defined by either the peptide or non-peptide ligands was equivalent.

Following the end of the incubation of washing (3 × 4 ml) in ice-cold NaCl, the assay was terminated by rapid filtration under vacuum through Whatman GF/B filter strips. The filters were transferred to vials containing 5 ml of Beckman HP scintillant and radioactivity was measured in a Packard series 5000 gamma counter. Binding results were analyzed using Graphpad INPLOT (Graphpad Software Inc.). Competition data were transformed by non-linear regression to obtain IC₅₀ values, which were then converted to K_i values by the Cheng-Prussoff equation.

Clog P determinations

Clog P represents the calculated log P value as determined from the PCMODELS programme (Daylight Chemical Information Systems).

Sources of drugs

Pentagastrin (Boc-β-alanine CCK tetrapeptide) was purchased from Cambridge Research Biochemicals or Peninsula

Laboratories. CCK_B antagonists were synthesized in the Medicinal Chemistry Department, Parke-Davis Neuroscience Research Centre (PDNRC), Cambridge, U.K., with the exception of PD141949, PD144598 and PD145942 which were synthesized in the Medicinal Chemistry Department, Warner-Lambert, Ann Arbor, Michigan, U.S.A.

Results

Tables 1 and 2 list the novel CCK_B ligands synthesized at PDNRC and used in the study, showing the side chain modifications to the core molecule. Synthesis of two CCK_B ligands of the pyrazolidinone series developed at Lilly, (compounds 20 and 17 in Howbert *et al.*, 1992) purported to show both good CCK_B binding and CCK_B/CCK_A selectivity, was undertaken to yield compounds 12 and 13 in the present study. The Merck, Sharp & Dohme CCK_B/gastrin antagonist L365260 was also screened (compound 20), thus providing a list of compounds derived from different chemical strategies.

The results described in this paper to novel CCK_B antagonists in the rat VMH were obtained from a total of 78 preparations. All of the compounds were evaluated on at least three separate occasions to provide equilibrium constant (K_e) values. These results are summarized, together with the data for binding affinities (K_i) for the same compounds, in Table 3 and the relationship between pK_i from binding studies and pK_e from the functional assay is shown graphically in Figure 1. The best fit line for the data had a slope of 1.0 and a coefficient of determination (R²) of 0.72.

Peptoid antagonists

Seventeen of the compounds tested were developed from the peptoid strategy, compounds exemplified by compound 2

Figure 1 Summary of pK values for CCK_B antagonists. Plotted on the ordinate scale are pK_i values derived from binding studies on mouse cortex, against pK_e values on the abscissa scale from the functional assay (rat VMH, with pentagastrin used as the agonist in the presence of devazepide). The data shown are for 20 compounds spanning five orders of magnitude in the two tests. The best fit line, indicated by the solid line, has a slope (m) of 1.00 and a coefficient of determination (R²) of 0.72. The dashed lines indicate the 95% confidence limits for the line.

Table 1 Chemical structures of 'peptoid' CCK_B ligands

R^1
 R^2
 Ar

Compound	R^1	R^2	Ar
(1)PD132461	CH ₂ OH	H	Ph
(2)PD134308	H	NHCO(CH ₂) ₂ CO ₂ H	Ph
(3)PD135158 ^a	H	NHCO(CH ₂) ₂ CO ₂ H	Ph
(4)PD135666	CH ₂ CO ₂ H	H	Ph
(5)PD135667	CH ₂ SCH ₂ CO ₂ H	H	Ph
(6)PD136450 ^b	H	NHCOCH:CHCO ₂ H	Ph
(7)PD137649	CH ₂ SO ₂ CH ₂ CO ₂ H	H	Ph
(8)PD140015	H	H	2-Pyridyl
(9)PD140376	CH ₂ CO ₂ H	H	4-Aminophenyl
(10)PD140548 ^c	CH ₂ CO ₂ H	H	Ph
(14)PD142308	CH ₂ CO ₂ H	H	3-Iodo-4-aminophenyl
(15)PD142898 ^d	CH ₂ CO ₂ H	H	Ph
(16)PD148101	CH ₂ OH	H	3-Pyridyl

^aN-terminal blocking group is 1S endobornylloxycarbonyl; ^b olefin has E configuration; ^cS stereochemistry at Trp centre and R configuration at homo-Phe α-carbon; ^dN-terminal blocking group is S,S-2-methylcyclohexyloxycarbonyl.

Table 2 Chemical structures of 'peptoid' CCK_B ligands

Compound
	R	
(11)PD141745	(S)Asp(S)PheNH ₂	
(17)PD141949
(18)PD144598
(19)PD145942

(PD134308, which is now called CI-988). In the present study the *K_i* for compound 2 against pentagastrin was 6.5 nM (range 0.8–10.4 nM), which agrees with previous reports showing this compound to be a powerful CCK_B antagonist (Hughes *et al.*, 1990). Compound 3 (PD135158) was also found to antagonize pentagastrin responses, with a *K_i* of 4.4 nM (range 1.7–7.4 nM). Both of the values differ slightly from those found using CCK as the agonist (Hughes *et al.*, 1990), possibly as a result of CCK_A receptor influence in the assays using the non-selective CCK agonist. Compounds 2 and 3 are carboxylic acids, as is compound 4 (PD135666), although this compound was somewhat less active in the

functional assay than would have been predicted from binding assays.

Compound 14(PD142308), which is the *m*-iodo *p*-amino phenyl derivative of compound 4, was the most powerful antagonist tested in the VMH assay. No difference was found between the *K_i* value obtained in receptor binding and its equivalent *K_e* value of 400 pM. Figure 2 shows typical data obtained using this compound in the rat VMH. Compound 5(PD135667) was less effective than might have been predicted in the functional assay. This compound has a high clog P value (5.8) but this is also found in compounds exhibiting a good correlation between binding and functional data from both the peptoid series e.g. compound 3 (PD135158) and entities from other classes e.g. compound 12. Another more pertinent structural feature of compound 5(PD135667) may be the inclusion of a sulphur-containing acid side chain. This feature is also found in compound 7(PD137649) which is the corresponding sulphone derivative, whereas compound 5(PD135667) has a thio-ether side chain moiety. Compound 7(PD137649) was also less effective in the functional test than would be expected.

Compound 10(PD140548) was tested as an example of a CCK_A receptor selective compound, having relatively low affinity for the CCK_B compared to the CCK_A binding site (compound 30p in Boden *et al.*, 1993). This compound still antagonized pentagastrin responses with a *K_e* in line with that expected, despite having only weak affinity for the CCK_B receptor. The same was true for compound 11(PD141745) which is a weak, non-selective CCK antagonist, characteristics which were exemplified in its relatively poor *pK_i* and *pK_e* values. Compound 15(PD142898) is a non-selective CCK antagonist with high affinity for both CCK_A and CCK_B receptors (compound 28 h in Boden *et al.*, 1993). The *pK_e* value obtained in the present study confirmed compound 15(PD142898) to be a powerful CCK_B antagonist.

CCK_B antagonists from other chemical strategies

Compounds from two other, differing, chemical classes were tested in the rat VMH model. Compounds 12, 13 and 20 were all CCK_B antagonists with *K_e* values in good agreement with those expected from the more extensive range of peptoid antagonists used in the present study.

Table 3 Summary of equilibrium constant (K_e) values and binding affinity constant values (K_i) for novel CCK_B antagonists

(N) PD number	K_e (nM)	K_e (range)	K_i (nM)	pK_e	pK_i
(1)PD132461	1.8	(0.8–3.3)	6.3	8.7	8.2
(2)PD134308	6.5	(1.8–10.4)	1.7	8.2	8.8
(3)PD135158	4.4	(1.7–7.4)	2.1	8.4	8.7
(4)PD135666	2.8	(0.1–8.4)	0.2	8.5	9.7
(5)PD135667	22.7	(19.0–28.0)	0.4	7.7	9.4
(6)PD136450	8.6	(6.1–9.7)	0.7	8.1	9.2
(7)PD137649	5.9	(3.2–10.3)	0.2	8.2	9.7
(8)PD140015	39.7	(10.1–83.0)	19.0	7.4	7.7
(9)PD140376	1.4	(0.8–2.0)	0.2	8.9	9.6
(10)PD140548	1150	(520–2500)	260.0	5.9	6.6
(11)PD141745	1200	(1010–1320)	1800	5.9	5.7
(12)	158.1	(100–235)	40.0	6.8	7.4
(13)	79.6	(27.3–162.3)	36.0	7.1	7.4
(14)PD142308	0.4	(0.3–0.5)	0.5	9.4	9.3
(15)PD142898	8.8	(5.4–10.8)	3.9	8.1	8.4
(16)PD148101	27.9	(23.4–31.2)	4.8	7.6	8.3
(17)PD141949	64.8	(53.7–102.7)	34.6	7.2	7.5
(18)PD144598	38.1	(17.2–64.1)	15.0	7.4	7.8
(19)PD145942	33.5	(25.2–41.7)	3.0	7.5	8.5
(20)	39.0	(33.6–43.8)	5.1	7.4	8.3

Functional assay for K_e value determination was the rat VMH with pentagastrin used as the agonist.

Binding assays were performed on the mouse cerebral cortex.

Compounds 12 and 13 are pyrazolidinone derivatives (compounds 20 and 17 in Howbert *et al.*, 1992). Compound 20 is L365260.

Discussion

The majority of the compounds shown in Table 1 were selected for evaluation in an electrophysiological assay on the basis of their high affinity and selectivity in the mouse cortex CCK_B receptor binding assay and their structural diversity. Comparison of functional data with results from binding assays revealed a highly positive correlation. One notable feature is that, irrespective of chemical class, there is a consistent difference between the absolute values obtained in the two tests, with binding values being around one-half an order of magnitude better than seen in the functional studies. Whether this is due to under-estimation of functional antagonism because of poor penetration of the compounds is not known. It is unlikely to be a result of insufficient equilibration time since compounds were always included for 30 min prior to construction of a second pentagastrin concentration-response curve. One other possible explanation would be that the binding of K_i values are over-estimates resulting from the use of a non-selective ligand for displacement experiments. The relatively low specific activity of [³H]-pentagastrin restricts the use of this radioligand in binding studies because of the possible errors which may be introduced with the requirement for higher tissue concentrations.

Included in the selection of compounds were examples of carboxylic acids e.g. compounds 2(PD134308), 3(PD135158) and 4(PD135666), pyridine bases e.g. compound 8 and 16 and neutral alcohols e.g. compounds 1(PD132461) and 19(PD145942). Log P values for the series span almost two orders of magnitude, from 4.1 (compound 8, PD140015) to 5.8 (compound 5, PD135667). The pK_a values for the acids is even more wide-ranging (4.16 for compound 6(PD136450) to 6.16 for compound 2(PD134308)). Despite this physicochemical diversity, only one compound 1(PD132461) yielded a pK_e value greater than its pK_i counterpart. The reason for this is unclear since compound 1(PD132461) is a neutral alcohol derivative similar to compounds 16 (PD148101) and 19 (PD145942) with an unexceptional log P value of 5.19.

In summary, the results presented herein from *in vitro* functional studies confirm all 20 compounds to be antagonists at CCK_B receptors in the rat brain. Thus, we can conclude that the VMH slice preparation provides a robust CNS assay and provides a good functional adjunct to obtain a rank order which mirrors that found in binding studies for novel CCK_B antagonists.

Figure 2 Ratemeter histogram showing typical trace obtained with one of the peptoid CCK_B antagonists: (a) shows the firing rate of a spontaneously active VMH neurone, given as firing rate (spikes 10 s⁻¹ epoch) against time course of the experiment along the abscissa scale in min. Pentagastrin was applied directly dissolved in the perfusing ACSF for 1 min (shown by ●) at the concentrations given to yield a control dose-response curve (● in b). Compound 14 was perfused for 30 min before repeating the pentagastrin dose-response curve in the continued presence of compound 14. The responses to 10 nM and 30 nM pentagastrin were reduced. After 1 h in the presence of compound 14, subsequent applications of increasing doses of the agonist were used to produce the data given by (■) in (b). The equilibrium constant for compound 14 in this experiment was 313 pM.

References

- BOCK, M.G. (1991). Development of non-peptide cholecystokinin type B receptor antagonists. *Drugs of the Future*, **16**, 631–640.
- BODEN, P.R., HIGGINBOTTOM, M., HILL, D.R., HORWELL, D.C., HUGHES, J., REES, D.C., ROBERTS, E., SINGH, L., SUMAN-CHAUHAN, N. & WOODRUFF, G.N. (1993). Cholecystokinin dipeptoid antagonists: design, synthesis and anxiolytic profile of some novel CCK-A and CCK-B selective and 'mixed' CCK-A/CCK-B antagonists. *J. Med. Chem.*, **36**, 552–565.
- BODEN, P. & HILL, R.G. (1987). Effects of cholecystokinin and pentagastrin on neuronal activity in the ventromedial nucleus of the rat hypothalamic slice preparation. *Br. J. Pharmacol.*, **92**, 585P.
- BODEN, P. & HILL, R.G. (1988). Effect of cholecystokinin and related peptides on neuronal activity in the ventromedial nucleus of the rat hypothalamus. *Br. J. Pharmacol.*, **94**, 246–252.
- BRADWEJN, J., KOSZYCKI, D. & METERRISSIAN, G. (1990). Cholecystokinin tetrapeptide induces panic attacks in patients with panic disorder. *Can. J. Psychiatry*, **35**, 83–85.
- CHANG, R.S.L. & LOTTI, V. (1986). Biochemical and pharmacological characterization of an extremely potent and selective non-peptide cholecystokinin antagonist. *Proc. Natl. Acad. Sci. U.S.A.*, **83**, 4923–4926.
- DOCKRAY, G.J. (1976). Immunochemical evidence for cholecystokinin-like peptides in brain. *Nature*, **264**, 568–570.
- DOURISH, C.T., RUCKERT, A.C., TATTERSALL, F.D. & IVERSEN, L.L. (1989). Evidence that decreased feeding induced by systemic injection of cholecystokinin is mediated by CCK_A receptors. *Eur. J. Pharmacol.*, **173**, 233–234.
- EVANS, B.E., BOCK, M.G., RITTLE, K.E., DIPARDO, R.M., WHITTER, W.L., VEBER, D.F., ANDERSON, P.S. & FREIDINGER, R.M. (1986). Design of potent, orally effective, nonpeptidic antagonists of the peptide hormone cholecystokinin. *Proc. Natl. Acad. Sci. U.S.A.*, **83**, 4918–4922.
- EVANS, B.E., RITTLE, K.E., BOCK, M.G., DIPARDO, R.M., FREIDINGER, R.M., WHITTER, W.L., LUNDELL, G.F., VEBER, D.F., ANDERSON, P.S., CHANG, R.S.L., LOTTI, V.J., CERINO, D.J., KLING, P.J., KUNKEL, K.A., SPRINGER, J.P. & HIRSHFELD, J. (1988). Methods for drug discovery: Development of potent, selective, orally effective cholecystokinin antagonists. *J. Med. Chem.*, **31**, 2235–2246.
- FARIS, P.L., KOMISURAK, B.R., WATKINS, L.R. & MAYER, D.J. (1983). Evidence for the neuropeptide cholecystokinin as an antagonist of opiate analgesia. *Science*, **219**, 310–312.
- GIBBS, J. & SMITH, G.P. (1973). Cholecystokinin-decreased food intake in rats. *J. Comp. Physiol.*, **84**, 488–495.
- HOWBERT, J.J., LOBB, K.L., BROWN, R.F., REEL, J.K., NEEL, D.A., MASON, N.R., MENDELSON, L.G., HODGKISS, J.P. & KELLY, J.S. (1992). A novel series of non-peptide CCK and gastrin antagonists: medicinal chemistry and electrophysiological demonstration of antagonism. In *Multiple Cholecystokinin Receptors – Progress Towards CNS Therapeutic Targets*. ed. Dourish, C., Cooper, S.J., Iversen, S.D. & Iversen, L.L. Oxford University Press: London.
- HORWELL, D.C. (1991). Development of CCK_B antagonists. *Neuropeptides*, **19** (Suppl.), 57–64.
- HUGHES, J., BODEN, P., COSTALL, B., DOMENEY, A., KELLY, E., HORWELL, D.C., HUNTER, J.C., PINNOCK, R.D. & WOODRUFF, G.N. (1990). Development of a class of cholecystokinin type B receptor antagonists having potent anxiolytic activity. *Proc. Natl. Acad. Sci. U.S.A.*, **87**, 6728–6732.
- INNIS, R.B., CORREA, F.M.A., UHL, G.R., SCHNEIDER, B. & SNYDER, S.H. (1979). Cholecystokinin octapeptide-like immunoreactivity: histochemical localization in rat brain. *Proc. Natl. Acad. Sci. U.S.A.*, **76**, 521–525.
- JURNA, I. & ZETLER, G. (1981). Antinociceptive effect of centrally administered caerulein and cholecystokinin (CCK). *Eur. J. Pharmacol.*, **73**, 323–331.
- LOTTI, V. & CHANG, R.S.L. (1989). A potent and selective non-peptide gastrin antagonist and brain (CCK_B) receptor ligand: L365,260. *Eur. J. Pharmacol.*, **162**, 273–280.
- PINNOCK, R.D., RICHARDSON, R.S., BODEN, P.R. & WOODRUFF, G.N. (1992). Cholecystokinin receptors in the rat brain *in vitro*: sensitivity to CCK_A and CCK_B receptor agonists and antagonists. *Mol. Neuropharmacol.*, **1**, 211–218.
- WATKINS, L.R., KINSHECK, I.B. & MAYER, D.J. (1983). Potentiation of opiate analgesia and apparent reversal of morphine tolerance by proglumide. *Science*, **224**, 395–396.
- WEISENFELD-HALLIN, Z., XU, X.-J., HUGHES, J., HORWELL, D.C. & HOKFELT, T. (1990). PD134308, a selective antagonist of cholecystokinin type B receptor, enhances the analgesic effect of morphine and synergistically interacts with intrathecal galanin to depress spinal nociceptive reflexes. *Proc. Natl. Acad. Sci. U.S.A.*, **87**, 7105–7109.
- WOODRUFF, G.N., HILL, D.R., BODEN, P.R., PINNOCK, R.D., SINGH, L. & HUGHES, J. (1991). Functional role of brain CCK receptors. *Neuropeptides*, **19** (Suppl.), 45–56.

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